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  *                W E L C O M E   T O   T H E                      *
  *          U. S.   P A T E N T   T E X T   F I L E                *
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(FILE 'USPAT' ENTERED AT 12:09:46 ON 24 FEB 1998)
L1       106470 S (INTERLEUKIN OR PROINTERLEUKIN)(3A)CONVERT? OR ICE
L2          315 S APOPTO?
L3          165 S L1 AND L2
L4            0 S CASPASE?
L5           45 S INTERLEUKIN(3A)CONVERT?
L6       111804 S (HOMOLOG? OR FAMIL?)
L7          357 S L1(P)L6
L8           15 S L3 AND L7

L3
1.  5,721,104, Feb. 24, 1998, Screening assay for anti-HIV drugs; Irvin S. Y.
Chen, et al., 435/7.1, 7.2, 7.21 [IMAGE AVAILABLE]

2.  5,721,103, Feb. 24, 1998, Trienoic retinoid compounds and methods; Marcus F.
Boehm, et al., 435/7.1; 514/532, 571, 617, 885, 886, 912; 560/55, 75; 562/402,
432, 450; 564/161, 170 [IMAGE AVAILABLE]

3.  5,719,195, Feb. 17, 1998, Treatment of psoriasis with 11-cis-retinoic acid;
Mark S. Braiman, 514/725 [IMAGE AVAILABLE]

4.  5,716,929, Feb. 10, 1998, Inhibitors of **interleukin**-1.beta.
**converting** enzyme; Guy W. Bemis, et al., 514/18, 19; 530/331; 548/217 [IMAGE
AVAILABLE]

5.  5,714,320, Feb. 3, 1998, Rolling circle synthesis of oligonucleotides and
amplification of select randomized circular oligonucleotides; Eric T. Kool,
435/6, 5, 91.1, 91.2; 536/23.1, 24.3, 24.32, 24.33, 24.5 [IMAGE AVAILABLE]

6.  5,714,170, Feb. 3, 1998, Method of inducing resistance to tumor growth;
Renato Baserga, et al., 424/573, 93.2, 93.21, 422, 423, 424, 425, 572; 435/91.1,
285.1; 536/23.1; 604/890.1 [IMAGE AVAILABLE]

7.  5,712,375, Jan. 27, 1998, Systematic evolution of ligands by exponential
enrichment: tissue selex; Kirk B. Jensen, et al., 530/412; 435/6, 91.2; 536/25.4
[IMAGE AVAILABLE]

8.  5,712,307, Jan. 27, 1998, Methods of inducing the production of hemoglobin
and treating pathologies associated with abnormal hemoglobin activity using
phemylacetic acids and derivatives therof; Dvorit Samid, 514/538, 563, 567
[IMAGE AVAILABLE]
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9. 5,712,117, Jan. 27, 1998, Cytoplasmic antiproteinase-2 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

10. 5,712,115, Jan. 27, 1998, Human cell death-associated protein; Phillip R. Hawkins, et al., 435/69.1, 320.1, 326; 536/23.5; 935/22, 66 [IMAGE AVAILABLE]

11. 5,710,178, Jan. 20, 1998, Compositions and methods for therapy and prevention of pathologies including cancer, AIDS, and anemia; Dvorit Samid, 514/557, 568, 570 [IMAGE AVAILABLE]

12. 5,710,159, Jan. 20, 1998, Integrin receptor antagonists; Matthew Ernst Voss, et al., 514/275, 303, 340, 341, 370, 386, 393, 394, 395, 397; 544/331; 546/118, 272.1; 548/190, 193, 194, 302.7, 304.7, 311.1 [IMAGE AVAILABLE]

13. 5,710,153, Jan. 20, 1998, Tetrazole compound; Kazuyuki Ohmoto, et al., 514/236.2, 255, 326, 340, 381, 382; 544/132, 366; 546/210, 268.4; 548/253 [IMAGE AVAILABLE]

14. 5,710,026, Jan. 20, 1998, Cytoplasmic antiproteinase-2 and cytoplasmic antiproteinase-3 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

15. 5,708,025, Jan. 13, 1998, Methods for promoting wound healing; Dvorit Samid, 514/538, 563, 567, 885, 886, 928 [IMAGE AVAILABLE]

16. 5,707,624, Jan. 13, 1998, Treatment of Kaposi's sarcoma by inhibition of scatter factor; Brian J. Nickoloff, et al., 424/158.1, 143.1, 145.1, 152.1 [IMAGE AVAILABLE]

17. 5,705,380, Jan. 6, 1998, Identification of a gene encoding TULP2, a retina specific protein; Michael North, et al., 435/6, 91.2, 320.1; 536/23.1, 23.5, 24.3, 24.31, 24.33; 935/6, 8, 77, 78 [IMAGE AVAILABLE]

18. 5,705,342, Jan. 6, 1998, Interaction of BCL-2 and R-RAS; James R. Bischoff, et al., 435/6, 7.1, 7.21, 7.23, 7.24, 375 [IMAGE AVAILABLE]

19. 5,700,821, Dec. 23, 1997, Phosphatase inhibitors and methods of use thereof; John S. Lazo, et al., 514/374, 255, 313, 314, 326, 340; 544/369; 546/167, 169, 171, 209, 271.4 [IMAGE AVAILABLE]

20. 5,700,638, Dec. 23, 1997, Cell death regulator; Stanley J. Korsmeyer, 435/6, 7.1, 7.2, 7.21, 7.31, 7.8, 69.1, 172.3; 436/501; 530/350 [IMAGE AVAILABLE]

21. 5,698,445, Dec. 16, 1997, Human PAK65; Arie Abo, et al., 435/325, 252.3, 320.1, 348; 536/23.2 [IMAGE AVAILABLE]

22. 5,698,428, Dec. 16, 1997, Human PAK65; Arie Abo, et al., 435/194 [IMAGE AVAILABLE]

23. 5,696,109, Dec. 9, 1997, Synthetic catalytic free radical scavengers useful as antioxidants for prevention and therapy of disease; Bernard Malfroy-Camine, et al., 514/185, 184, 492, 501, 502, 505 [IMAGE AVAILABLE]

24. 5,695,944, Dec. 9, 1997, Modulation of bcl-2 phosphorylation; Carlo M. Croce, et al., 435/7.21, 7.23; 436/63, 64, 813 [IMAGE AVAILABLE]

25. 5,693,627, Dec. 2, 1997, Use of phosphotyrosine phosphatase inhibitors for controlling cellular proliferation; Gary L. Schieven, 514/137; 435/184, 244; 514/141; 564/12; 568/14, 17 [IMAGE AVAILABLE]

26. 5,693,474, Dec. 2, 1997, Methods for cancer diagnosis and prognosis; Jerry Shay, et al., 435/6, 15, 91.2, 183, 184, 194; 436/63, 64; 935/77, 78 [IMAGE AVAILABLE]

27. 5,691,341, Nov. 25, 1997, **Apoptosis** regulating composition; Satoru Nakai, et al., 514/254, 249, 250, 251, 255 [IMAGE AVAILABLE]

28. 5,691,179, Nov. 25, 1997, Cell death regulators; Stanley J. Korsmeyer, 435/172.3, 252.3, 254.11, 320.1; 536/23.5, 24.31 [IMAGE AVAILABLE]

29. 5,688,935, Nov. 18, 1997, Nucleic acid ligands of tissue target; Andrew Stephens, et al., 536/23.1; 435/6, 91.2; 935/77, 78 [IMAGE AVAILABLE]

30. 5,688,915, Nov. 18, 1997, Long term maintenance of lymphocytes in vitro; Yakov Ron, et al., 530/380; 435/41 [IMAGE AVAILABLE]

31. 5,688,773, Nov. 18, 1997, Method of selectively destroying neoplastic cells; E. Antonio Chiocca, et al., 514/44; 424/93.1, 93.21; 435/172.3, 320.1 [IMAGE AVAILABLE]

32. 5,688,690, Nov. 18, 1997, Human cytotoxic lymphocyte signal transduction surface protein (P38) and monoclonal antibodies thereto; Nicholas M. Valiante, et al., 435/334, 343, 343.2; 530/388.1, 388.75 [IMAGE AVAILABLE]

33. 5,686,598, Nov. 11, 1997, Genes associated with retinal dystrophies; Michael North, et al., 536/23.5 [IMAGE AVAILABLE]

34. 5,686,072, Nov. 11, 1997, Epitope-specific monoclonal antibodies and immunotoxins and uses thereof; Jonathan W. Uhr, et al., 424/183.1; 435/7.24; 530/388.73, 391.7 [IMAGE AVAILABLE]

35. 5,684,222, Nov. 4, 1997, Mutant mouse having a disrupted TNFRp55; Tak W. Mak, 800/2; 435/172.3; 800/DIG.1, DIG.2 [IMAGE AVAILABLE]

36. 5,681,820, Oct. 28, 1997, Guanidinoalkyl glycine .beta.-amino acids useful for inhibiting tumor metastasis; Peter Gerrard Ruminski, 514/18, 20, 340, 357, 616 [IMAGE AVAILABLE]

37. 5,681,589, Oct. 28, 1997, Liposomal ceramide-related liposomes and the therapeutic use thereof; Yong Wei, et al., 424/450; 428/402.2 [IMAGE AVAILABLE]

38. 5,679,541, Oct. 21, 1997, Programmed cell death antagonist protein; Nancy M. Bonini, et al., 435/69.1, 252.3, 320.1; 530/300, 350; 536/22.1, 23.1, 23.5, 24.3, 24.31 [IMAGE AVAILABLE]

US PAT NO: 5,712,115 [IMAGE AVAILABLE] L3: 10 of 165
SUMMARY: BSUM(4)Normal . . . in cell death. Cysteine proteases are among known molecules which carry out genetically programmed cell death. Molecules such as CPP32, **apoptotic** protein; Ced-3, cell death protein; and **ICE**, **interleukin**-1 B-**converting** enzyme are directly associated with cell destruction. These molecules are all effectors in receptor-mediated cascades which lead to cell destruction.

BSUM(8) The . . . and mediator death domains was strengthened by recent experiments in which the cloning and transient expression of death domains induced **apoptosis**. Where any of the death domains of either receptors or mediators were overexpressed, self-association of the death domains triggered **apoptosis**. Specific mutations such as the replacement of I.sub.225 with alanine in the death domain of mouse Fas/APO1 prohibits binding of. . . the cysteine protease inhibitor crmA from cowpox virus into cells transfected with mediators or their death domains prevents binding and **apoptosis**.

BSUM(17) The . . . syndrome, systemic lupus erythematosus, and thyroiditis. Also formulated CDAP can be delivered to metastatic cells, cancers or tumors to induce **apoptosis** of those cells.

DETDESC: DETD(3) The . . . L.sub.115 and ends at L.sub.172. Thus, CDAP appears to be a undescribed intracellular mediator in the receptor-mediated cascade which governs **apoptosis**.

DETD(8)The . . . T lymphocytes. Other pharmaceutical compositions such as formulated CDAP can be delivered to metastatic cells, cancers or tumors to provoke **apoptosis** of those cells.

DETD(60) CDAP . . . engineered to give high expression (Stanger, supra). Using a microscope and Hoescht 33258 stain, those nuclei which express CDAP appear **apoptotic**. Control cells transfected with a vector containing only an antibiotic resistance gene or .beta. galactosidase appear normal. More elaborate assays. . .

DETD(71) The . . . the protein itself, provides opportunities for early intervention in cancers. In these metastatic, tumor or cancer cells, excess CDAP induces **apoptosis**. On the other hand, excessive natural CDAP has been correlated with unnecessary tissue destruction in autoimmune conditions such as rheumatoid. . .

DETD(72)Because CDAP is a regulatory molecule in the signalling pathway which induces **apoptosis**, designing a PNA which binds in the area of the leucine zipper may prevent intracellular movement or interaction with the nuclear chromatin. This, in turn, compromises the **apoptotic** activity of CDAP in diseases, listed above, where excessive numbers of T lymphocytes cause irreversible tissue destruction

DETD(94)Polynucleotide . . . diagnosis of conditions or diseases or monitoring treatment of conditions or diseases where the expression of CDAP causes wasting or **apoptosis**. For example, polynucleotide sequences encoding CDAP may be used in hybridization or PCR assays of fluids or tissues from biopsies. . .

DETD(100) The . . . used to shrink or eradicate metastatic cells, cancers or tumors. In such instances, the expression of the introduced cdap induces **apoptosis**.

DETD(166) CDAP . . . buffer containing X-gal to visualize .beta. galactosidase activity. Using a microscope and Hoescht 33258 stain, the nuclei expressing CDAP appear **apoptotic**: the affected cells show loss of adherence, are intensely blue and shrunken, and exhibit membrane blebbing. Control cells are transfected. . .

L5
1. 5,716,929, Feb. 10, 1998, Inhibitors of **interleukin**-1.beta. **converting** enzyme; Guy W. Bemis, et al., 514/18, 19; 530/331; 548/217 [IMAGE AVAILABLE]

2. 5,714,484, Feb. 3, 1998, .alpha.-(1,3-dicarbonylenol ether) methyl ketones as cysteine protease inhibitors; Mary P. Zimmerman, et al., 514/231.5, 459, 460, 471; 544/149, 152; 549/292, 318, 417 [IMAGE AVAILABLE]

3. 5,712,117, Jan. 27, 1998, Cytoplasmic antiproteinase-2 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

4. 5,712,115, Jan. 27, 1998, Human cell death-associated protein; Phillip R. Hawkins, et al., 435/69.1, 320.1, 326; 536/23.5; 935/22, 66 [IMAGE AVAILABLE]

5. 5,710,153, Jan. 20, 1998, Tetrazole compound; Kazuyuki Ohmoto, et al., 514/236.2, 255, 326, 340, 381, 382; 544/132, 366; 546/210, 268.4; 548/253 [IMAGE AVAILABLE]

6. 5,710,026, Jan. 20, 1998, Cytoplasmic antiproteinase-2 and cytoplasmic antiproteinase-3 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

7. 5,705,380, Jan. 6, 1998, Identification of a gene encoding TULP2, a retina specific protein; Michael North, et al., 435/6, 91.2, 320.1; 536/23.1, 23.5, 24.3, 24.31, 24.33; 935/6, 8, 77, 78 [IMAGE AVAILABLE]

8. 5,696,157, Dec. 9, 1997, Sulfonated derivatives of 7-aminocoumarin; Hui-Ying Wang, et al., 514/457; 549/285, 288, 289 [IMAGE AVAILABLE]

9. 5,693,847, Dec. 2, 1997, Heteroatom functionalized .alpha.-methyl ketones; Roger D. Tung, et al., 560/16; 549/518, 552 [IMAGE AVAILABLE]

10. 5,686,598, Nov. 11, 1997, Genes associated with retinal dystrophies; Michael North, et al., 536/23.5 [IMAGE AVAILABLE]

US PAT NO: 5,712,115 [IMAGE AVAILABLE] L5: 4 of 45
SUMMARY: BSUM(4) Normal development, differentiation, and defense in multicellular organisms involves the activation and expression of genes which ultimately result in cell death. Cysteine proteases are among known molecules which carry out genetically programmed cell death. Molecules such as CPP32, apoptotic protein; Ced-3, cell death protein; and ICE, **interleukin**-1 B-**converting** enzyme are directly associated with cell destruction. These molecules are all effectors in receptor-mediated cascades which lead to cell destruction.

US PAT NO: 5,705,380 [IMAGE AVAILABLE] L5: 7 of 45
SUMMARY: BSUM(65)A number of apoptotic and anti-apoptotic genes are expressed in neurons and photoreceptors, and may be involved in retinal degeneration. These cells depend on factors such as nerve growth factor and brain derived neurotrophic factor for survival, and may undergo apoptosis where the factor or its receptor are mutated. Among the anti-apoptotic genes of interest are bcl-2, bcl-xL and mcl-1. Inducers of apoptosis include fas (CD95), rnyc, bax, bcl-xs, TNF receptor and the family of cysteine proteases that includes **interleukin** 1 .beta.-**converting** enzyme.

US PAT NO: 5,686,598 [IMAGE AVAILABLE] L5: 10 of 45
SUMMARY: BSUM(65) A number of apoptotic and anti-apoptotic genes are expressed in neurons and photoreceptors, and may be involved in retinal degeneration. These cells depend on factors such as nerve growth factor and brain derivedneurotrophic factor for survival, and may undergo apoptosis where the factor or its receptor are mutated. Among the anti-apoptotic genes of interest are bcl-2, bcl-xL and mcl-1. Inducers of apoptosis include fas (CD95), myc, bax, bcl-xs, TNF receptor and the family of cysteine proteases that includes **interleukin** 1 .beta.-**converting** enzyme.

L8
1. 5,716,929, Feb. 10, 1998, Inhibitors of **interleukin**-1.beta. **converting** enzyme; Guy W. Bemis, et al., 514/18, 19; 530/331; 548/217 [IMAGE AVAILABLE]

2. 5,712,117, Jan. 27, 1998, Cytoplasmic antiproteinase-2 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

3. 5,710,026, Jan. 20, 1998, Cytoplasmic antiproteinase-2 and cytoplasmic antiproteinase-3 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

4. 5,705,380, Jan. 6, 1998, Identification of a gene encoding TULP2, a retina specific protein; Michael North, et al., 435/6, 91.2, 320.1; 536/23.1, 23.5, 24.3, 24.31, 24.33; 935/6, 8, 77, 78 [IMAGE AVAILABLE]

5. 5,686,598, Nov. 11, 1997, Genes associated with retinal dystrophies; Michael North, et al., 536/23.5 [IMAGE AVAILABLE]

US PAT NO: 5,716,929 [IMAGE AVAILABLE] L8: 1 of 15
TITLE: Inhibitors of **interleukin**-1.beta. **converting** enzyme

ABSTRACT:
The present invention relates to novel classes of compounds which are inhibitors of **interleukin**-1.beta. **converting** enzyme. The **ICE** inhibitors of this invention are characterized by specific structural and physicochemical features. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for inhibiting **ICE** activity and consequently, may be advantageously used as agents against interleukin-1 mediated diseases, including inflammatory diseases, autoimmune diseases and neurodegenerative diseases. This invention also relates to methods for inhibiting **ICE** activity and methods for treating interleukin-1 mediated diseases using the compounds and compositions of this invention.

SUMMARY: BSUM(2)
The present invention relates to novel classes of compounds which are inhibitors of **interleukin**-1.beta. **converting** enzyme ("**ICE**"). The **ICE** inhibitors of this invention are characterized by specific structural and physicochemical features. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for inhibiting **ICE** activity and consequently, may be advantageously used as agents against interleukin-1 ("IL-I") mediated diseases, including inflammatory diseases, autoimmune diseases and neurodegenerative diseases. This invention also relates

to methods for inhibiting **ICE** activity and methods for treating interleukin-1 mediated diseases using the compounds and compositions of this invention.

BSUM(5) IL-1.beta. . . . is not processed by a signal peptidase. March, C. J., Nature, 315, pp. 641-647 (1985). Instead, pIL-1.beta. is cleaved by **interleukin**-1.beta. **converting** enzyme ("**ICE**") between Asp-116 and Ala-117 to produce the biologically active C-terminal fragment found in human serum and synovial fluid. Sleath, P.. . . J. Biol. Chem., 265, pp. 14526-14528 (1992); A. D. Howard et al., J. Immunol., 147, pp. 2964-2969 (1991). Processing by **ICE** is also necessary for the transport of mature IL-1.beta. through the cell membrane.

BSUM(6) **ICE** is a cysteine protease localized primarily in monocytes. It converts precursor IL-1.beta. to the mature form. Black, R. A. et. . . FEBS Lett., 247, pp. 386-390 (1989); Kostura, M. J. et al., Proc. Natl. Acad. Sci. USA, 86, pp. 5227-5231 (1989). **ICE**, or its **homologues**, also appears to be involved in the regulation of cell death or **apoptosis**. Yuan, J. et al., Cell, 75, pp. 641-652 (1993); Miura, M. et al., Cell, 75, pp. 653-660 (1993); Nett-Fiordalisi, M. A. et al., J. Cell Biochem., 17B, p. 117 (1993). In particular, **ICE** or **ICE****homologues** are thought to be associated with the regulation of **apoptosis** in neurogenerative diseases, such as Alzheimer's and Parkinson's disease. Marx, J. and M. Baringa, Science, 259, pp. 760-762 (1993); Gagliardini,. . .

BSUM(7) **ICE** has been previously described as a heterodimer composed of two subunits, p20 and p10 (20 kDa and 10 kDa molecular. . . p30 form, through an activation mechanism that is autocatalytic. Thornberry, N. A. et al., Nature, 356, pp. 768-774 (1992). The **ICE** proenzyme has been divided into several functional domains: a prodomain (p14), a p22/20 subunit, a polypeptide linker and a p10. . .

BSUM(8) Full . . . 94/00154. The p20 and p10 cDNA and amino acid sequences are also known. Thornberry et al., supra. Murine and rat **ICE** have also been sequenced and cloned. They have high amino acid and nucleicacid sequence **homology** to human **ICE**. Miller, D. K. et al., Ann. N.Y. Acad. Sci., 696, pp. 133-148 (1993); Molineaux, S. M. et al., Proc. Nat. Acad. Sci., 90, pp. 1809-1813 (1993). Knowledge of the primary structure of **ICE**, however, does not allow prediction of its tertiary structure. Nor does it afford an understanding of the structural, conformational and chemical interactions of **ICE** and its substrate pIL-1.beta. or other substrates or inhibitors.

BSUM(9) **ICE** inhibitors represent a class of compounds useful for the control of inflammation or **apoptosis** or both. Peptide and peptidyl inhibitors of **ICE** have been described. PCT patent applications WO 91/15577; WO 93/05071; WO 93/09135; WO 93/14777 and WO 93/16710; and European patent. . .

BSUM(10)Accordingly, the need exists for compounds that can effectively inhibit the action of **ICE**, for use as agents for preventing and treating chronic and acute forms of IL-1 mediated diseases, including various cancers, as. . .

BSUM(12) The present inve ntion provides novel classes of compounds, andpharmaceutically acceptable derivatives thereof, that are useful as inhibitors of **ICE**. These compounds can be used alone or in combination with other therapeutic or prophylactic agents, such as antibiotics, immunomodulators or. . . IL-1. According to a preferred embodiment, the compounds of this invention are capable of binding to theactive site of **ICE** and inhibiting the activity of that enzyme.

BSUM(13) It is a principal object of this invention to provide novel classes of inhibitors of **ICE**. These novel classes of **ICE** inhibitors are characterized by the following structural and physicochemical features:

BSUM(14) a) . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of **ICE**, said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(15)b) . . . first and a second moderately hydrophobic moiety, said moieties each being capable of associating with a separate binding pocket of **ICE** when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(16) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of **ICE**.

BSUM(17) It is also an object of this invention to provide a method for identification, design or prediction of **ICE** inhibitors comprising the steps of:

BSUM(19) b) determining a low-energy conformation for binding of said compound to the active site of **ICE**;

BSUM(20) c) . . . in said conformation to form at least two hydrogen bonds with the non-carbon backbone atoms of Arg-341 and Ser-339 of **ICE**;

BSUM(21)d) evaluating the capability of said compound in said conformation to associate with at least two of the binding pockets of **ICE** selected from the group consisting of the P2 binding pocket, the P3 binding pocket, the P4 binding pocket and the. . .

BSUM(22)e) evaluating the capability of said compound in said conformation to interact with the P1 binding pocket of **ICE**; and

BSUM(23) f) accepting or rejecting said candidate compound as an **ICE** inhibitor based on the determinations and evaluations carried out in the preceding steps.

BSUM(24) It is a further object of this invention to provide novel classes of **ICE** inhibitors represented by formulas: ##STR1##

BSUM(29) The term "active site" refers to any or all of the following sites in **ICE**: the substrate binding site, the site where an inhibitor binds and the site where the cleavage of substrate occurs. The. . .

BSUM(30) The . . . "S subsite", "S pocket", and the like, refer to binding subsites, or portions of the substrate binding site on the **ICE** molecule. The amino acid residues of the substrate are given designations according to their position relative to the scissile bond,. . . residues of the substrate are also labeled P1, P1', etc., by analogy with the substrate. The binding subsites of the **ICE** molecule which receive the residues labeled P1, P1', etc., are designated S1, S1', etc., or may alternately be designated "the. . .

BSUM(31) The terms "P2 binding pocket" or "S2 subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues Pro-290, Val-338 or Trp-340.

BSUM(32) The terms "P3 binding pocket" or "S3 subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues Pro-177, Arg-178, Thr-180, Arg-341 or. . .

BSUM(33) The terms "P4 binding pocket" or "S4 subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues His-342, Met-345, Val-348, Arg-352, Asp-381,. . .

BSUM(34) The terms "P1 binding pocket" or "S1 subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues Arg-179, His-237, Gln-283, or Arg-341.

BSUM(35) The terms "P' binding pocket" or "S' subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues Phe-173, Ile-176, His-237, Gly-238, Ile-239,. . .

BSUM(45) The term "association" is used in reference to a condition of proximity between an inhibitor or portions thereof to an **ICE** molecule or portions thereof wherein the juxtaposition is energetically favored by electrostatic or van der Waals interactions.

BSUM(50) The term "scaffold" refers to a structural building block which forms the basis of an **ICE** inhibitor according to this invention. Various moieties and functional groups are intended to be appended to the scaffold. The scaffolds of this invention are thus depicted having open valences. Various scaffolds of **ICE** inhibitors according to this invention include the portions: ##STR2## In those scaffolds, the NH and CO or SO.sub.2 moieties represent. . . a second hydrogen bonding moiety, said moieties each being capable of forming a hydrogen bond with a backbone atom of **ICE**, said backbone atom being selected from the group consisting of the carbonyl oxyg

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*        WELCOME TO THE          *
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(FILE 'USPAT' ENTERED AT 12:09:46 ON 24 FEB 1998)
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L8         15 S L3 AND L7
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L3

1. 5,721,104, Feb. 24, 1998, Screening assay for anti-HIV drugs; Irvin S. Y. Chen, et al., 435/7.1, 7.2, 7.21 [IMAGE AVAILABLE]

2. 5,721,103, Feb. 24, 1998, Trienoic retinoid compounds and methods; Marcus F. Boehm, et al., 435/7.1; 514/532, 571, 617, 885, 886, 912; 560/55, 75; 562/402, 432, 450; 564/161, 170 [IMAGE AVAILABLE]

3. 5,719,195, Feb. 17, 1998, Treatment of psoriasis with 11-cis-retinoic acid; Mark S. Braiman, 514/725 [IMAGE AVAILABLE]

4. 5,716,929, Feb. 10, 1998, Inhibitors of **interleukin**-1.beta. **converting** enzyme; Guy W. Bemis, et al., 514/18, 19; 530/331; 548/217 [IMAGE AVAILABLE]

5. 5,714,320, Feb. 3, 1998, Rolling circle synthesis of oligonucleotides and amplification of select randomized circular oligonucleotides; Eric T. Kool, 435/6, 5, 91.1, 91.2; 536/23.1, 24.3, 24.32, 24.33, 24.5 [IMAGE AVAILABLE]

6. 5,714,170, Feb. 3, 1998, Method of inducing resistance to tumor growth; Renato Baserga, et al., 424/573, 93.2, 93.21, 422, 423, 424, 425, 572; 435/91.1, 285.1; 536/23.1; 604/890.1 [IMAGE AVAILABLE]

7. 5,712,375, Jan. 27, 1998, Systematic evolution of ligands by exponential enrichment: tissue selex; Kirk B. Jensen, et al., 530/412; 435/6, 91.2; 536/25.4 [IMAGE AVAILABLE]

8. 5,712,307, Jan. 27, 1998, Methods of inducing the production of hemoglobin and treating pathologies associated with abnormal hemoglobin activity using phemylacetic acids and derivatives therof; Dvorit Samid, 514/538, 563, 567 [IMAGE AVAILABLE]

9. 5,712,117, Jan. 27, 1998, Cytoplasmic antiproteinase-2 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

10. 5,712,115, Jan. 27, 1998, Human cell death-associated protein; Phillip R. Hawkins, et al., 435/69.1, 320.1, 326; 536/23.5; 935/22, 66 [IMAGE AVAILABLE]

11. 5,710,178, Jan. 20, 1998, Compositions and methods for therapy and prevention of pathologies including cancer, AIDS, and anemia; Dvorit Samid, 514/557, 568, 570 [IMAGE AVAILABLE]

12. 5,710,159, Jan. 20, 1998, Integrin receptor antagonists; Matthew Ernst Voss, et al., 514/275, 303, 340, 341, 370, 386, 393, 394, 395, 397; 544/331; 546/118, 272.1; 548/190, 193, 194, 302.7, 304.7, 311.1 [IMAGE AVAILABLE]

13. 5,710,153, Jan. 20, 1998, Tetrazole compound; Kazuyuki Ohmoto, et al., 514/236.2, 255, 326, 340, 381, 382; 544/132, 366; 546/210, 268.4; 548/253 [IMAGE AVAILABLE]

14. 5,710,026, Jan. 20, 1998, Cytoplasmic antiproteinase-2 and cytoplasmic antiproteinase-3 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

15. 5,708,025, Jan. 13, 1998, Methods for promoting wound healing; Dvorit Samid, 514/538, 563, 567, 885, 886, 928 [IMAGE AVAILABLE]

16. 5,707,624, Jan. 13, 1998, Treatment of Kaposi's sarcoma by inhibition of scatter factor; Brian J. Nickoloff, et al., 424/158.1, 143.1, 145.1, 152.1 [IMAGE AVAILABLE]

17. 5,705,380, Jan. 6, 1998, Identification of a gene encoding TULP2, a retina specific protein; Michael North, et al., 435/6, 91.2, 320.1; 536/23.1, 23.5, 24.3, 24.31, 24.33; 935/6, 8, 77, 78 [IMAGE AVAILABLE]

18. 5,705,342, Jan. 6, 1998, Interaction of BCL-2 and R-RAS; James R. Bischoff, et al., 435/6, 7.1, 7.21, 7.23, 7.24, 375 [IMAGE AVAILABLE]

19. 5,700,821, Dec. 23, 1997, Phosphatase inhibitors and methods of use thereof; John S. Lazo, et al., 514/374, 255, 313, 314, 326, 340; 544/369; 546/167, 169, 171, 209, 271.4 [IMAGE AVAILABLE]

20. 5,700,638, Dec. 23, 1997, Cell death regulator; Stanley J. Korsmeyer, 435/6, 7.1, 7.2, 7.21, 7.31, 7.8, 69.1, 172.3; 436/501; 530/350 [IMAGE AVAILABLE]

21. 5,698,445, Dec. 16, 1997, Human PAK65; Arie Abo, et al., 435/325, 252.3, 320.1, 348; 536/23.2 [IMAGE AVAILABLE]

22. 5,698,428, Dec. 16, 1997, Human PAK65; Arie Abo, et al., 435/194 [IMAGE AVAILABLE]

23. 5,696,109, Dec. 9, 1997, Synthetic catalytic free radical scavengers useful as antioxidants for prevention and therapy of disease; Bernard Malfroy-Camine, et al., 514/185, 184, 492, 501, 502, 505 [IMAGE AVAILABLE]

24. 5,695,944, Dec. 9, 1997, Modulation of bcl-2 phosphorylation; Carlo M. Croce, et al., 435/7.21, 7.23; 436/63, 64, 813 [IMAGE AVAILABLE]

25. 5,693,627, Dec. 2, 1997, Use of phosphotyrosine phosphatase inhibitors for controlling cellular proliferation; Gary L. Schieven, 514/137; 435/184, 244; 514/141; 564/12; 568/14, 17 [IMAGE AVAILABLE]

26. 5,693,474, Dec. 2, 1997, Methods for cancer diagnosis and prognosis; Jerry Shay, et al., 435/6, 15, 91.2, 183, 184, 194; 436/63, 64; 935/77, 78 [IMAGE AVAILABLE]

27. 5,691,341, Nov. 25, 1997, **Apoptosis** regulating composition; Satoru Nakai, et al., 514/254, 249, 250, 251, 255 [IMAGE AVAILABLE]

28. 5,691,179, Nov. 25, 1997, Cell death regulators; Stanley J. Korsmeyer, 435/172.3, 252.3, 254.11, 320.1; 536/23.5, 24.31 [IMAGE AVAILABLE]

29. 5,688,935, Nov. 18, 1997, Nucleic acid ligands of tissue target; Andrew Stephens, et al., 536/23.1; 435/6, 91.2; 935/77, 78 [IMAGE AVAILABLE]

30. 5,688,915, Nov. 18, 1997, Long term maintenance of lymphocytes in vitro; Yakov Ron, et al., 530/380; 435/41 [IMAGE AVAILABLE]

31. 5,688,773, Nov. 18, 1997, Method of selectively destroying neoplastic cells; E. Antonio Chiocca, et al., 514/44; 424/93.1, 93.21; 435/172.3, 320.1 [IMAGE AVAILABLE]

32. 5,688,690, Nov. 18, 1997, Human cytotoxic lymphocyte signal transduction surface protein (P38) and monoclonal antibodies thereto; Nicholas M. Valiante, et al., 435/334, 343, 343.2; 530/388.1, 388.75 [IMAGE AVAILABLE]

33. 5,686,598, Nov. 11, 1997, Genes associated with retinal dystrophies; Michael North, et al., 536/23.5 [IMAGE AVAILABLE]

34. 5,686,072, Nov. 11, 1997, Epitope-specific monoclonal antibodies and immunotoxins and uses thereof; Jonathan W. Uhr, et al., 424/183.1; 435/7.24; 530/388.73, 391.7 [IMAGE AVAILABLE]

35. 5,684,222, Nov. 4, 1997, Mutant mouse having a disrupted TNFRp55; Tak W. Mak, 800/2; 435/172.3; 800/DIG.1, DIG.2 [IMAGE AVAILABLE]

36. 5,681,820, Oct. 28, 1997, Guanidinoalkyl glycine .beta.-amino acids useful for inhibiting tumor metastasis; Peter Gerrard Ruminski, 514/18, 20, 340, 357, 616 [IMAGE AVAILABLE]

37. 5,681,589, Oct. 28, 1997, Liposomal ceramide-related liposomes and the therapeutic use thereof; Yong Wei, et al., 424/450; 428/402.2 [IMAGE AVAILABLE]

38. 5,679,541, Oct. 21, 1997, Programmed cell death antagonist protein; Nancy M. Bonini, et al., 435/69.1, 252.3, 320.1; 530/300, 350; 536/22.1, 23.1, 23.5, 24.3, 24.31 [IMAGE AVAILABLE]

US PAT NO: 5,712,115 [IMAGE AVAILABLE] L3: 10 of 165

SUMMARY: BSUM(4)Normal . . . in cell death. Cysteine proteases are among known molecules which carry out genetically programmed cell death. Molecules such as CPP32, **apoptotic** protein; Ced-3, cell death protein; and **ICE**, **interleukin**-1 B-**converting** enzyme are directly associated with cell destruction. These molecules are all effectors in receptor-mediated cascades which lead to cell destruction.

BSUM(8) The . . . and mediator death domains was strengthened by recent experiments in which the cloning and transient expression of death domains induced **apoptosis**. Where any of the death domains of

either receptors or mediators were overexpressed, self-association of the death domains triggered **apoptosis**. Specific mutations such as the replacement of l.sub.225 with alanine in the death domain of mouse Fas/APO1 prohibits binding of. . . the cysteine protease inhibitor crmA from cowpox virus into cells transfected with mediators or their death domains prevents binding and **apoptosis**.

BSUM(17) The . . . syndrome, systemic lupus erythematosus, and thyroiditis. Also formulated CDAP can be delivered to metastatic cells, cancers or tumors to induce **apoptosis** of those cells.

DETDESC: DETD(3) The . . . L.sub.115 and ends at L.sub.172. Thus, CDAP appears to be a undescribed intracellular mediator in the receptor-mediated cascade which governs **apoptosis**.

DETD(8)The . . . T lymphocytes. Other pharmaceutical compositions such as formulated CDAP can be delivered to metastatic cells, cancers or tumors to provoke **apoptosis** of those cells.

DETD(60) CDAP . . . engineered to give high expression (Stanger, supra). Using a microscope and Hoescht 33258 stain, those nuclei which express CDAP appear **apoptotic**. Control cells transfected with a vector containing only an antibiotic resistance gene or .beta. galactosidase appear normal. More elaborate assays. .

DETD(71) The . . . the protein itself, provides opportunities for early intervention in cancers. In these metastatic, tumor or cancer cells, excess CDAP induces **apoptosis**. On the other hand, excessive natural CDAP has been correlated with unnecessary tissue destruction in autoimmune conditions such as rheumatoid. . .

DETD(72)Because CDAP is a regulatory molecule in the signalling pathway which induces **apoptosis**, designing a PNA which binds in the area of the leucine zipper may prevent intracellular movement or interaction with the nuclear chromatin. This, in turn, compromises the **apoptotic** activity of CDAP in diseases, listed above, where excessive numbers of T lymphocytes cause irreversible tissue destruction

DETD(94)Polynucleotide . . . diagnosis of conditions or diseases or monitoring treatment of conditions or diseases where the expression of CDAP causes wasting or **apoptosis**. For example, polynucleotide sequences encoding CDAP may be used in hybridization or PCR assays of fluids or tissues from biopsies. . .

DETD(100) The . . . used to shrink or eradicate metastatic cells, cancers or tumors. In such instances, the expression of the introduced cdap induces **apoptosis**.

DETD(166) CDAP . . . buffer containing X-gal to visualize .beta. galactosidase activity. Using a microscope and Hoescht 33258 stain, the nuclei expressing CDAP appear **apoptotic**: the affected cells show loss of adherence, are intensely blue and shrunken, and exhibit membrane blebbing. Control cells are transfected. .

L5

1. 5,716,929, Feb. 10, 1998, Inhibitors of **interleukin**-1.beta. **converting** enzyme; Guy W. Bemis, et al., 514/18, 19; 530/331; 548/217 [IMAGE AVAILABLE]

2. 5,714,484, Feb. 3, 1998, .alpha.-(1,3-dicarbonylenol ether) methyl ketones as cysteine protease inhibitors; Mary P. Zimmerman, et al., 514/231.5, 459, 460, 471; 544/149, 152; 549/292, 318, 417 [IMAGE AVAILABLE]

3. 5,712,117, Jan. 27, 1998, Cytoplasmic antiproteinase-2 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

4. 5,712,115, Jan. 27, 1998, Human cell death-associated protein; Phillip R. Hawkins, et al., 435/69.1, 320.1, 326; 536/23.5; 935/22, 66 [IMAGE AVAILABLE]

5. 5,710,153, Jan. 20, 1998, Tetrazole compound; Kazuyuki Ohmoto, et al., 514/236.2, 255, 326, 340, 381, 382; 544/132, 366; 546/210, 268.4; 548/253 [IMAGE AVAILABLE]

6. 5,710,026, Jan. 20, 1998, Cytoplasmic antiproteinase-2 and cytoplasmic antiproteinase-3 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

7. 5,705,380, Jan. 6, 1998, Identification of a gene encoding TULP2, a retina specific protein; Michael North, et al., 435/6, 91.2, 320.1; 536/23.1, 23.5, 24.3, 24.31, 24.33; 935/6, 8, 77, 78 [IMAGE AVAILABLE]

8. 5,696,157, Dec. 9, 1997, Sulfonated derivatives of 7-aminocoumarin; Hui-Ying Wang, et al., 514/457; 549/285, 288, 289 [IMAGE AVAILABLE]

9. 5,693,847, Dec. 2, 1997, Heteroatom functionalized .alpha.-methyl ketones; Roger D. Tung, et al., 560/16; 549/518, 552 [IMAGE AVAILABLE]

10. 5,686,598, Nov. 11, 1997, Genes associated with retinal dystrophies; Michael North, et al., 536/23.5 [IMAGE AVAILABLE]

US PAT NO: 5,712,115 [IMAGE AVAILABLE] L5: 4 of 45
SUMMARY: BSUM(4) Normal development, differentiation, and defense in multicellular organisms involves the activation and expression of genes which ultimately result in cell death. Cysteine proteases are among known molecules which carry out genetically programmed cell death. Molecules such as CPP32, apoptotic protein; Ced-3, cell death protein; and ICE, **interleukin**-1 B-**converting** enzyme are directly associated with cell destruction. These molecules are all effectors in receptor-mediated cascades which lead to cell destruction.

US PAT NO: 5,705,380 [IMAGE AVAILABLE] L5: 7 of 45
SUMMARY: BSUM(65)A number of apoptotic and anti-apoptotic genes are expressed in neurons and photoreceptors, and may be involved in retinal degeneration. These cells depend on factors such as nerve growth factor and brain derived neurotrophic factor for survival, and may undergo apoptosis where the factor or its receptor are mutated. Among the anti-apoptotic genes of interest are bcl-2, bcl-xL and mcl-1. Inducers of apoptosis include fas (CD95), myc, bax, bcl-xs, TNF receptor and the family of cysteine proteases that includes **interleukin** 1 .beta.-**converting** enzyme.

US PAT NO: 5,686,598 [IMAGE AVAILABLE] L5: 10 of 45
SUMMARY: BSUM(65) A number of apoptotic and anti-apoptotic genes are expressed in neurons and photoreceptors, and may be involved in retinal degeneration. These cells depend on factors such as nerve growth factor and brain derivedneurotrophic factor for survival, and may undergo apoptosis where the factor or its receptor are mutated. Among the anti-apoptotic genes of interest are bcl-2, bcl-xL and mcl-1. Inducers of apoptosis include fas (CD95), myc, bax, bcl-xs, TNF receptor and the family of cysteine proteases that includes **interleukin** 1 .beta.-**converting** enzyme.

L8

1. 5,716,929, Feb. 10, 1998, Inhibitors of **interleukin**-1.beta. **converting** enzyme; Guy W. Bemis, et al., 514/18, 19; 530/331; 548/217 [IMAGE AVAILABLE]

2. 5,712,117, Jan. 27, 1998, Cytoplasmic antiproteinase-2 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

3. 5,710,026, Jan. 20, 1998, Cytoplasmic antiproteinase-2 and cytoplasmic antiproteinase-3 and coding sequences; Cindy A. Sprecher, 435/69.2, 252.3, 254.11, 320.1, 325; 530/413; 536/23.5 [IMAGE AVAILABLE]

4. 5,705,380, Jan. 6, 1998, Identification of a gene encoding TULP2, a retina specific protein; Michael North, et al., 435/6, 91.2, 320.1; 536/23.1, 23.5, 24.3, 24.31, 24.33; 935/6, 8, 77, 78 [IMAGE AVAILABLE]

5. 5,686,598, Nov. 11, 1997, Genes associated with retinal dystrophies; Michael North, et al., 536/23.5 [IMAGE AVAILABLE]

US PAT NO: 5,716,929 [IMAGE AVAILABLE] L8: 1 of 15
TITLE: Inhibitors of **interleukin**-1.beta. **converting** enzyme
ABSTRACT: The present invention relates to novel classes of compounds which are inhibitors of **interleukin**-1.beta. **converting** enzyme. The **ICE** inhibitors of this invention are characterized by specific structural and physicochemical features. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for inhibiting **ICE** activity and consequently, may be advantageously used as agents against interleukin-1 mediated diseases, including inflammatory diseases, autoimmune diseases and neurodegenerative diseases. This invention also relates to methods for inhibiting **ICE** activity and methods for treating interleukin-1 mediated diseases using the compounds and compositions of this invention.

SUMMARY: BSUM(2) The present invention relates to novel classes of compounds which are inhibitors of **interleukin**-1.beta. **converting** enzyme (**ICE**). The **ICE** inhibitors of this invention are characterized by specific structural and physicochemical features. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for inhibiting **ICE** activity and consequently, may be advantageously used as agents against interleukin-1 ("IL-I") mediated diseases, including inflammatory diseases, autoimmune diseases and neurodegenerative diseases. This invention also relates to methods for inhibiting **ICE** activity and methods for treating interleukin-1 mediated diseases using the compounds and compositions of this invention.

BSUM(5) IL-1.beta. . . . is not processed by a signal peptidase. March, C. J., Nature, 315, pp. 641-647 (1985). Instead, pIL-1.beta. is cleaved by **interleukin**-1.beta. **converting** enzyme (**ICE**) between Asp-116 and Ala-117 to produce the biologically active C-terminal fragment found in human serum and synovial fluid. Sleath, P.. . . J. Biol. Chem., 265, pp. 14526-14528 (1992); A. D. Howard et al., J. Immunol., 147, pp. 2964-2969 (1991). Processing by **ICE** is also necessary for the transport of mature IL-1.beta. through the cell membrane.

BSUM(6) **ICE** is a cysteine protease localized primarily in monocytes. It converts precursor IL-1.beta. to the mature form. Black, R. A. et. . . FEBS Lett., 247, pp. 386-390 (1989); Kostura, M. J. et al., Proc. Natl. Acad. Sci. USA, 86, pp. 5227-5231 (1989). **ICE**, or its **homologues**, also appears to be involved in the regulation of cell death or **apoptosis**. Yuan, J. et al., Cell, 75, pp. 641-652 (1993); Miura, M. et al., Cell, 75, pp. 653-660 (1993); Nett-Fiordalisi, M. A. et al., J. Cell Biochem., 17B, p. 117 (1993). In particular, **ICE** or **ICE****homologues** are thought to be associated with the regulation of **apoptosis** in neurogenerative diseases, such as Alzheimer's and Parkinson's disease. Marx, J. and M. Baringa, Science, 259, pp. 760-762 (1993); Gagliardini,. . .

BSUM(7) **ICE** has been previously described as a heterodimer composed of two subunits, p20 and p10 (20 kDa and 10 kDa molecular. . . p30 form, through an activation mechanism that is autocatalytic. Thornberry, N. A. et al., Nature, 356, pp. 768-774 (1992). The **ICE** proenzyme has been divided into several functional domains: a prodomain (p14), a p22/20 subunit, a polypeptide linker and a p10. . .

BSUM(8) Full . . . 94/00154. The p20 and p10 cDNA and amino acid sequences are also known. Thornberry et al., supra. Murine and rat **ICE** have also been sequenced and cloned. They have high amino acid and nucleicacid sequence **homology** to human **ICE**. Miller, D. K. et al., Ann. N.Y. Acad. Sci., 696, pp. 133-148 (1993); Molineaux, S. M. et al., Proc. Nat. Acad. Sci., 90, pp. 1809-1813 (1993). Knowledge of the primary structure of **ICE**, however, does not allow prediction of its tertiary structure. Nor does it afford an understanding of the structural, conformational and chemical interactions of **ICE** and its substrate pIL-1.beta. or other substrates or inhibitors.

BSUM(9) **ICE** inhibitors represent a class of compounds useful for the control of inflammation or **apoptosis** or both. Peptide and peptidyl inhibitors of **ICE** have been described. PCT patent applications WO 91/15577; WO 93/05071; WO 93/09135; WO 93/14777 and WO 93/16710; and European patent. . .

BSUM(10)Accordingly, the need exists for compounds that can effectively inhibit the action of **ICE**, for use as agents for preventing and treating chronic and acute forms of IL-1 mediated diseases, including various cancers, as. . .

BSUM(12) The present inve ntion provides novel classes of compounds, andpharmaceutically acceptable derivatives thereof, that are useful as inhibitors of **ICE**. These compounds can be used alone or in

combination with other therapeutic or prophylactic agents, such as antibiotics, immunomodulators or. . . IL-1. According to a preferred embodiment, the compounds of this invention are capable of binding to theactive site of **ICE** and inhibiting the activity of that enzyme.

BSUM(13) It is a principal object of this invention to provide novel classes of inhibitors of **ICE**. These novel classes of **ICE** inhibitors are characterized by the following structural and physicochemical features:

BSUM(14) a) . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of **ICE**, said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(15)b) . . . first and a second moderately hydrophobic moiety, said moieties each being capable of associating with a separate binding pocket of **ICE** when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(16) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of **ICE**.

BSUM(17) It is also an object of this invention to provide a method for identification, design or prediction of **ICE** inhibitors comprising the steps of:

BSUM(19) b) determining a low-energy conformation for binding of said compound to the active site of **ICE**;

BSUM(20) c) . . . in said conformation to form at least two hydrogen bonds with the non-carbon backbone atoms of Arg-341 and Ser-339 of **ICE**;

BSUM(21)d) evaluating the capability of said compound in said conformation to associate with at least two of the binding pockets of **ICE** selected from the group consisting of the P2 binding pocket, the P3 binding pocket, the P4 binding pocket and the. . .

BSUM(22)e) evaluating the capability of said compound in said conformation to interact with the P1 binding pocket of **ICE**; and

BSUM(23) f) accepting or rejecting said candidate compound as an **ICE** inhibitor based on the determinations and evaluations carried out in the preceding steps.

BSUM(24) It is a further object of this invention to provide novel classes of **ICE** inhibitors represented by formulas: ##STR1##

BSUM(29) The term "active site" refers to any or all of the following sites in **ICE**: the substrate binding site, the site where an inhibitor binds and the site where the cleavage of substrate occurs. The. . .

BSUM(30) The . . . "S subsite", "S pocket", and the like, refer to binding subsites, or portions of the substrate binding site on the **ICE** molecule. The amino acid residues of the substrate are given designations according to their position relative to the scissile bond,. . . residues of the substrate are also labeled P1, P1', etc., by analogy with the substrate. The binding subsites of the **ICE** molecule which receive the residues labeled P1, P1', etc., are designated S1, S1', etc., or may alternately be designated "the. . .

BSUM(31) The terms "P2 binding pocket" or "S2 subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues Pro-290, Val-338 or Trp-340.

BSUM(32) The terms "P3 binding pocket" or "S3 subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues Pro-177, Arg-178, Thr-180, Arg-341 or. . .

BSUM(33) The terms "P4 binding pocket" or "S4 subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues His-342, Met-345, Val-348, Arg-352, Asp-381,. . .

BSUM(34) The terms "P1 binding pocket" or "S1 subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues Arg-179, His-237, Gln-283, or Arg-341.

BSUM(35) The terms "P' binding pocket" or "S' subsite" of the **ICE** active site are equivalent and are defined as the space surrounded by amino acid residues Phe-173, Ile-176, His-237, Gly-238, Ile-239,. . .

BSUM(45) The term "association" is used in reference to a condition of proximity between an inhibitor or portions thereof to an **ICE** molecule or portions thereof wherein the juxtaposition is energetically favored by electrostatic or van der Waals interactions.

BSUM(50) The term "scaffold" refers to a structural building block which forms the basis of an **ICE** inhibitor according to this invention. Various moieties and functional groups are intended to be appended to the scaffold. The scaffolds of this invention are thus depicted having open valences. Various scaffolds of **ICE** inhibitors according to this invention include the portions: ##STR2## In those scaffolds, the NH and CO or SO.sub.2 moieties represent. . . a second hydrogen bonding moiety, said moieties each being capable of forming a hydrogen bond with a backbone atom of **ICE**, said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-of Arg-341,. . .

BSUM(51) The . . . of a hydrogen bonding moiety which is capable of forming a hydrogen bond with the carbonyl oxygen of Arg-341 of **ICE** or the carbonyl oxygen of Ser-339 of **ICE** are excluded from substitution. These excluded hydrogen atoms include those which comprise an --NH-- group which is alpha to a. . .

BSUM(53) The . . . to a numerical measure of the effectiveness of a compound in inhibiting the activity of a target enzyme such as **ICE**. Lower values of K.sub.i reflect higher effectiveness. The K.sub.i value is a derived by fitting experimentally determined rate data to. . .

BSUM(55) The . . . difference between the free conformation energy of a compound and the bound conformation energy of that compound when bound to **ICE**. The strain energy can be determined by the following steps: Evaluate the energy of the molecule when it has the conformation necessary for binding to **ICE**. Then minimize and reevaluate the energy--this is the free conformation energy. The strain energy for binding of a potential inhibitor to **ICE** is the difference between the free conformation energy and the bound conformation energy. In a preferred embodiment, the strain energy. . .

BSUM(59) The . . . which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an anti-**ICE** active metabolite or residue thereof.

BSUM(62) The **ICE** inhibitors of this invention may contain one or more "asymmetric" carbon atoms and thus may occur as racemates and racemic. . .

BSUM(63) The **ICE** inhibitors of this invention may comprise ring structures which may optionally be substituted at carbon, nitrogen or other atoms by. . .

BSUM(67) We have discovered that compounds possessing the following novel combination of features are surprisingly effective **ICE** inhibitors:

BSUM(68) a) . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of **ICE**, said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(69) b) . . . first and a second moderately hydrophobic moiety, said moieties each being capable of associating with a separate binding pocket of **ICE** when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(70) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of **ICE**.

BSUM(71) Preferably, any moderately hydrophobic moiety associating with the P2 binding pocket of **ICE** does so in such a way that:

BSUM(72) a) . . . center of mass of the moderately hydrophobic moiety in the P2 binding pocket to the carbonyl oxygen of Arg-341 of **ICE** is between about 7.1 .ANG. and about 12.5 .ANG.;

BSUM(73) b) . . . center of mass of the moderately hydrophobic moiety in the P2 binding pocket to the amide nitrogen of Arg-341 of **ICE** is between about 6.0 .ANG. and about 12 .ANG.; and

BSUM(74) c) . . . center of mass of the moderately hydrophobic moiety in the P2 binding pocket to the carbonyl oxygen of Ser-339 of **ICE** is between about 3.7 .ANG. and about 9.5 .ANG..

BSUM(75) Preferably, any moderately hydrophobic moiety associating with the P3 binding pocket of **ICE** does so in such a way that:

BSUM(76)a) . . . center of mass of the moderately hydrophobic moiety in the P3 binding pocket to the carbonyl oxygen of Arg-341 of **ICE** is between about 3.9 .ANG. and about 9.5 .ANG.;

BSUM(77) b) . . . center of mass of the moderately hydrophobic moiety in the P3 binding pocket to the amide nitrogen of Arg-341 of **ICE** is between about 5.4 .ANG. and about 11 .ANG.; and

BSUM(78) c) . . . center of mass of the moderately hydrophobic moiety in the P3 binding pocket to the carbonyl oxygen of Ser-339 of **ICE** is between about 7.0 .ANG. and about 13 .ANG..

BSUM(79) Preferably, any moderately hydrophobic moiety associating with the P4binding pocket of **ICE** does so in such a way that:

BSUM(80) a) . . . center of mass of the moderately hydrophobic moiety in the P4 binding pocket to the carbonyl oxygen of Arg-341 of **ICE** is between about 4.5 .ANG. and about 7.5 .ANG.;

BSUM(81) b) . . . center of mass of the moderately hydrophobic moiety in the P4 binding pocket to the amide nitrogen of Arg-341 of **ICE** is between about 5.5 .ANG. and about 8.5 .ANG.; and

BSUM(82) c) . . . center of mass of the moderately hydrophobic moiety in the P4 binding pocket to the carbonyl oxygen of Ser-339 of **ICE** is between about 8 .ANG. and about 11 .ANG..

BSUM(83) Preferably, any moderately hydrophobic moiety associating with the P' binding pocket of **ICE** does so in such a way that:

BSUM(84) a) . . . center of mass of the moderately hydrophobic moiety in the P' binding pocket to the carbonyl oxygen of Arg-341 of **ICE** is between about 11 .ANG. and about 16 .ANG.;

BSUM(85) b) . . . center of mass of the moderately hydrophobic moiety in the P' binding pocket to the amide nitrogen of Arg-341 of **ICE** is between about 10 .ANG. and about 15 .ANG.; and

BSUM(86) c) . . . center of mass of the moderately hydrophobic moiety in the P' binding pocket to the carbonyl oxygen of Ser-339 of **ICE** is between about 8 .ANG. and about 12 .ANG..

BSUM(88) The . . . inhibitors of the present invention. These same means may be used to select a candidate compound for screening as an **ICE** inhibitor. This design or selection may begin with selection of the various moieties which fill binding pockets.

BSUM(103) Using . . . expensive experimentation to determine enzymatic inhibition activity of particular compounds. The method also is useful to facilitate rational design of **ICE** inhibitors and therapeutic andprophylactic agents against IL-1-mediated diseases. Accordingly, the present invention relates to such inhibitors.

BSUM(104) A . . . to carry out each of the above evaluations as well as the evaluations necessary in screening a candidate compound for **ICE** inhibiting activity. Generally, these techniques involve determining the location and binding proximity of a given moiety, the occupied space of. . .

BSUM(105) Different classes of active **ICE** inhibitors, according to this invention, may interact in similar ways with the various binding pockets of the **ICE** active site. The spatial arrangement of these important groups is often referred to as a pharmacophore. The concept of the. . .

BSUM(106) Different classes of **ICE** inhibitors of this invention may also [redacted] different scaffolds or core structures, but all of these cores will allow the. . . ability to match the pharmacophore, i.e., their structural identity relative to the shape and properties of the active site of **ICE**.

BSUM(107) The **ICE** inhibitors of one embodiment of this invention comprise a first and a second hydrogen bonding moiety, a first and a. . .

BSUM(108) The **ICE** inhibitors of another embodiment (A) of this invention are those of formula .alpha.: ##STR4## wherein: X.sub.1 is CH or N;

BSUM(166) The **ICE** inhibitors of another embodiment (B) of this invention are those of formula .alpha.: ##STR11## wherein: X.sub.1 is --CH;

BSUM(466) The **ICE** inhibitors of another embodiment (C) of this invention are represented by the formula .sigma.: ##STR85## wherein the ring is optionally. . .

BSUM(511) The **ICE** inhibitors of another embodiment (D) of this invention are represented by the formula .pi.: ##STR90## each T.sub.1 is independently selected. . .

BSUM(550) The **ICE** inhibitors of another embodiment (E) of this invention are represented by formula v: ##STR94## wherein: m is 0, 1, or. . .

BSUM(596) The **ICE** inhibitors of another embodiment (F) of this invention are represented by formula .delta.: ##STR101## wherein: R.sub.1 is R.sub.5 –(A).sub.p –;

BSUM(631) The **ICE** inhibitors of this invention may be synthesized using conventional techniques. Advantageously, these compounds are conveniently synthesized from readily available starting. . .

BSUM(632) The compounds of this invention are among the most readily synthesized **ICE** inhibitors known. Previously described **ICE** inhibitors often contain four or more chiral centers and numerous peptide linkages. The relative ease with which the compounds of. . .

BSUM(637) The compounds of this invention are excellent ligands for **ICE**. Accordingly, these compounds are capable of targeting and inhibiting events in IL-1 mediated diseases, such as the conversion of precursor. .. neurodegenerative diseases. For example, the compounds of this invention inhibit the conversion of precursor IL-1.beta. to mature IL-1.beta. by inhibiting **ICE**. Because **ICE** is essential for the production of mature IL-1, inhibition of that enzyme effectively blocksinitiation of IL-1 mediated physiological effects. . .

BSUM(639) Alternatively, . . . compositions either alone or together with other compounds of this invention in a manner consistent with the conventional utilization of **ICE** inhibitors in pharmaceutical compositions. For example, a compound of this invention may be combined with pharmaceutically acceptable adjuvants conventionally employed. . .

BSUM(640) The compounds of this invention may also be co-administered with other **ICE** inhibitors to increase the effect of therapy or prophylaxis against various IL-1-mediated diseases.

BSUM(643) When . . . to the patient. Alternatively, pharmaceutical or prophylactic compositions according to this invention may be comprised of a combination of an **ICE** inhibitor of this invention and another therapeutic or prophylactic agent.

BSUM(652) Inflammatory . . . sclerosis. And target neurodegenerative diseases include, for example, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and primary lateral sclerosis. The **ICE** inhibitors of this invention may also be used to promote wound healing. And the **ICE** inhibitors of this invention may be used to treat infectious diseases.

BSUM(654) The compounds of this invention are also useful as commercial reagents which effectively bind to **ICE** or other cysteine proteases. As commercial reagents, the compounds of this invention, and their derivatives, may be used to block. . .

DETDESC: DETD(4) Pick 2 hydrogen bonding moieties of **ICE**, here, the backbone C.dbd.O and N--H of Arg-341.

DETD(6) Pick . . . is performed by using molecular mechanics techniques to minimize the scaffold fragment in the context of the active site of **ICE**. ##STR109## Step 3)

DETD(10) Link . . . minimum number of bonds consistent with a chemically reasonable structure. Minimize the entire composite molecule in the active site of **ICE**. ##STR113## Step 7)

DETD(11) Evaluate the energy of the molecule when it has the conformation necessary for binding to **ICE**. Then minimize and reevaluate the energy--this is the free conformation energy. The strain energy for binding of the potential inhibitor to **ICE** is the difference between the free conformation energy and the bound conformation energy. The strain energy should be less than. . .

DETD(18)
65 .mu.l buffer (10 mM Tris, 1 mM DTT, 0.1% CHAPS @pH 8.1)
10 .mu.l **ICE** (50 nM final concentration to give a rate of .sup..about. 1mOD/min)
5 .mu.l DMSO/Inhibitor mixture
20 .mu.l. . .

DETD(19) The visible **ICE** assay is run in a 96-well microtiter plate. Buffer, **ICE** and DMSO (if inhibitor is present) are added to the wells in the order listed. The components are left to. . .

DETD(22)
65 .mu.l buffer (10 mM Tris, 1 mM DTT, 0.1% CHAPS @pH 8.1)
10 .mu.l **ICE** (2-10 nM final concentration)
5 .mu.l DMSO/inhibitor solution
20 .mu. 150 .mu.M Substrate (30 .mu.M final)
100 .mu.l. . .

DETD(23) The assay is run in a 96 well [redacted]ter plate. Buffer and **ICE** are added to the wells. The components are left to incubate at 37.degree. C. for 15 minutes in a temperature-controlled. .

DETD(27) Processing of pre-IL-1.beta. by **ICE** can be measured in cell culture using a variety of cell sources. Human PBMC obtained from healthy donors provides a. . .

DETD(29) An . . . 0.1% for all drug dilutions. A concentration titration which brackets the apparent K.sub.i for a test compound determined in an **ICE** inhibition assay is generally used for the primary compound screen.

DETD(74) To a cooled solution (**ice** bath) of N-tert-Butoxycarbonyl-4- phenoxyproline methyl ester (0.6 g) in 20 ml of ethyl acetate was bubbled anhydrous hydrogen chloride until saturated.. . .

DETD(120) 4-[3-(4-Hydroxyphenyl)propyl]imidazole-2-carboxylic . . . C., the mixture was warmed to 25.degree. C. and stirred for 16 h. The reaction mixture was cooled in an **ice** bath and quenched with a dropwise addition of water (20 ml). The resulting mixture was briefly stirred at 25.degree. C.. . .

DETD(163) 5-Benzylpyrrole-2-carboxylic . . . major part of the ethanol was removed and the remaining liquid was diluted with water, washed with ether, cooled in **ice** and acidified with concentrated hydrochloric acid. The mixture was extracted with ether. The combined extracts were washed with brine, dried. . .

DETD(164) (2R,S,3S)N.sup.2 . . . dry DMF (10 ml). Pyrrole-2-carboxylic acid (34a) (244 mg, 2.2 mmol) was added and the solution was cooled in an **ice** bath before the addition of N,N-diisopropylamine (0.78 g, 6.0 mmol), 1-hydroxybenzotriazole (0.54 g, 4.0 mmol) and ethyl dimethylaminopropyl carbodiimide hydrochloride. . .

DETD(186) Methyl[6-benzyl-3-benzyloxycarbonylamino-1,2-dihydro-2-oxo-1-pyridyl]- acetate . . . methyl bromoacetate (2.5 ml, 26.2 mmol) and kept for 3 h. The resulting mixture was poured onto a mixture of **ice** and 1M HCl. The resulting solid was filtered off then dissolved in dichloromethane. The resulting solution was dried (MgSO.sub.4), decolourized. . .

DETD(301) N-tert-Butoxycarbonyl . . . 80 mmol) was added to the mixture and refluxed for 6 hours. The mixture was quenched with the addition of **ice** chips, then additional water was added and the mixture was washed with hexane. The aqueous layer was acidified with 10%. . .

DETD(401) Insofar as the compounds of this invention are able to inhibit **ICE** in vitro and furthermore, may be delivered orally to mammals, they are of evident clinical utility for the treatment of IL-1 mediated diseases. These tests are predictive of the compounds ability to inhibit **ICE** in vivo.

CLAIMS: 59. A pharmaceutical composition for inhibiting an **ICE**-mediated function comprising a pharmaceutically effective amount of a compound according to any one of claims 1-22, 23-32 and 33-54 and. . .

US PAT NO: 5,712,117 [IMAGE AVAILABLE] L8: 2 of 15
ABSTRACT: Cytoplasmic . . . proteins encoded thereby are useful in the purification of proteins and in the treatment of inflammatory diseases and diseases involving **apoptosis**.

SUMMARY: BSUM(4) **Interleukin**-1.beta. **converting** enzyme (**ICE**) is an another example of a cysteine protease that plays an important role in inflammation. **ICE** is responsible for the activation of interleukin-1.beta., which is a critical cytokine in the inflammatory process. Serpins which inhibit **ICE** may therefore play an important role in inflammation. One such serpin is a viral protein encoded by the cowpox virus crmA gene. It is believed that expression of crmA protein inhibits **ICE** and thereby blocks migration of inflammatory cells in cowpox lesions. (See Ray, C. A., et. al. (1992) Cell 69:597-604.) Isolated cellular serpins that inhibit **ICE** in a similar manner to the crmA protein can be useful in the modulation of the inflammatory response. Agents that. . .

BSUM(5) **ICE** is but one member of a **family** of serine proteases that play important roles in normal physiology and in pathophysiology. For example, another member of the **ICE** **family**, Ich-1, is involved in regulation of **apoptosis**. Furthermore, evidence is accumulating that regulation of **apoptosis** plays a role in a variety of different diseases, including cancer. Therefore, isolated serpin molecules which inhibit Ich-1 could be used to regulate **apoptosis** and treat a number of diseases.

DETDESC: DETD(2) The . . . acid and protein compositions are also useful in the treatment of inflammatory diseases and in the treatment of diseases involving **apoptosis**. In addition, these compositions can be used in in vitro diagnostic procedures for these diseases.

DETD(8) The . . . trypsin-like specificity. The biological activity of CAP-3 can be determined, for example, by its ability to inhibit proteases in the **ICE** **family**. Particular protein modifications considered minor would include substitution of amino acids of similar chemical properties, e.g., glutamic acid for aspartic. . .

DETD(45) The . . . herein by reference. As an additional example, specificity for inhibition of other proteases of interest, such as those in the **ICE** **family**, is determined by adding CAP-2 and CAP-3 to preparations of these enzymes in vitro and monitoring changes in enzyme activity.

DETD(46) Pharmacological . . . to those of skill in the art. For example, CAP-2 or CAP-3 may inhibit inflammation by inhibiting the activity of **ICE** or other serine proteases involved in the inflammation process. A number of in vitro and animal model systems are used. . . reference. The effect of CAP-2 or CAP-3 and antagonists or agonists thereof can be demonstrated by other methods, e..g, transfecting **Ice**, Ich-1L, or other gene which induces programmed cell death or cell degeneration (e.g., ced-3) into a cell which is also. . .

DETD(47) The . . . be an endogenous inhibitor of specific trypsin-like serine proteases, and CAP-3 may be an endogenous inhibitor for members of the **ICE** **family** of proteases including Ich-1. Proteases with trypsin-like specificity are involved in many physiologically important processes, and **ICE** and Ich-1 play important roles in inflammation and **apoptosis**, respectively. Because of this, determination of CAP-2 or CAP-3 in biological samples can be useful in medicine.

DETD(78) The crmA protein functions as a specific inhibitor of **ICE** which represents a prototype of a larger **family** of **ICE**-like **homologs**. The **ICE** **family** of cysteine proteinases have been linked to both the negative and positive regulation of **apoptosis**. A human **homolog** of **ICE** has been identified and designated as Ich-1. In contrast to **ICE**, Ich-1-mediated effects on **apoptosis** of Rat-1 cells is only partially blocked by either microinjected or coexpressed crmA protein. These findings suggest that

Ich-1 and the crmA serpin interact weakly and further suggests that Ich-1 and **ICE** have distinct but overlapping substrate specificities.

US PAT NO: 5,710,026 [IMAGE AVAILABLE] L8: 3 of 15
ABSTRACT: Cytoplasmic . . . proteins encoded thereby are useful in the purification of proteins and in the treatment of inflammatory diseases and diseases involving **apoptosis**.

SUMMARY: BSUM(4)**Interleukin**-1.beta. **converting** enzyme (**ICE**) is an another example of a cysteine protease that plays an important role in inflammation. **ICE** is responsible for the activation of interleukin-1.beta., which is a critical cytokine in the inflammatory process. Serpins which inhibit **ICE** may therefore play an important role in inflammation. One such serpin is a viral protein encoded by the cowpox virus crmA gene. It is believed that expression of crmA protein inhibits **ICE** and thereby blocks migration of inflammatory cells in cowpox lesions. (See Ray, C. A., et. al. (1992) Cell 69:597-604.) Isolated cellular serpins that inhibit **ICE** in a similar manner to the crmA protein can be useful in the modulation of the inflammatory response. Agents that. . .

BSUM(5)*ICE** is but one member of a **family** of serine proteases that play important roles in normal physiology and in pathophysiology. For example, another member of the **ICE** **family**, Ich-1, is involved inregulation of **apoptosis**. Furthermore, evidence is accumulating that regulation of **apoptosis** plays a role in a variety of different diseases, including cancer. Therefore, isolated serpin molecules which inhibit Ich-1 could be used to regulate **apoptosis** and treat a number of diseases.

BSUM(14) The . . . acid and protein compositions are also useful in the treatment of inflammatory diseases and in the treatment of diseases involving **apoptosis**. In addition, these compositions can be used in in vitro diagnostic procedures for these diseases.

BSUM(20) The . . . trypsin-like specificity. The biological activity of CAP-3 can be determined, for example, by its ability to inhibit proteases in the **ICE** **family**. Particular protein modifications considered minor would include substitution of amino acids of similar chemical properties, e.g., glutamic acid for aspartic. . .

BSUM(57) The . . . herein by reference. As an additional example, specificity for inhibition of other proteases of interest, such as those in the **ICE** **family**, is determined by adding CAP-2 and CAP-3 to preparations of these enzymes in vitro and monitoring changes in enzyme activity.

BSUM(58) Pharmacological . . . to those of skill in the art. For example, CAP-2 or CAP-3 may inhibit inflammation by inhibiting the activity of **ICE** or other serine proteases involved in the inflammation process. A number of in vitro and animal model systems are used. . . reference. The effect of CAP-2 or CAP-3 and antagonists or agonists thereof can be demonstrated by other methods, e.g., transfecting **Ice**, Ich-1L, or other gene which induces programmed cell death or cell degeneration (e.g., ced-3) into a cell which is also. . .

BSUM(59) The . . . be an endogenous inhibitor of specific trypsin-like serine proteases, and CAP-3 may be an endogenous inhibitor for members of the **ICE** **family** of proteases including Ich-1. Proteases with trypsin-like specificity are involved in many physiologically important processes, and **ICE** and Ich-1 play important roles in inflammation and **apoptosis**, respectively. Because of this, determination of CAP-2 or CAP-3 in biological samples can be useful in medicine.

DETDESC: DETD(23) The crmA protein functions as a specific inhibitor of **ICE** which represents a prototype of a larger **family** of **ICE**-like **homologs**. The **ICE** **family** of cysteine proteinases have been linked to both the negative and positive regulation of **apoptosis**. A human **homolog** of **ICE** has been identified and designated as Ich-1. In contrast to **ICE**, Ich-1-mediated effects on **apoptosis** of Rat-1 cells is only partially blocked by either microinjected or coexpressed crmA protein. These findings suggest that Ich-1 and the crmA serpin interact weakly and further suggests that Ich-1 and **ICE** have distinct but overlapping substrate specificities.

US PAT NO: 5,705,380 [IMAGE AVAILABLE] L8: 4 of 15
SUMMARY: BSUM(61) The . . . proteins are associated with degeneration of specific neural cells, e.g. photoreceptors, in the retina. The disease histology is consistent with **apoptosis** of affected cells. The availability of the subject gene sequences provides a means of analyzing the biology and biochemistry of. . .

BSUM(62) A pathways of particular interest is photoreceptor **apoptosis**. Mutations in the .beta. subunit of cGMP phosphodiesterase cause retinal degeneration in mice with the rd1 mutation and in humans, and in rd1/rd1 mice an abnormal accumulation of cGMP appears to trigger **apoptosis** of the photoreceptor cells.

BSUM(63)Drug . . . assays, genetic complementation, etc. There are a number of characterized genes and gene products that operate to regulate or effect **apoptosis**.

BSUM(64) Complementation in animal and yeast models is particularly useful in the study of **apoptosis**. The genetics of programmed cell death has been well-defined in several animal models. Both C. elegans and D. melanogaster regulate **apoptosis** through the expression of two gene products, ced-3 and ced-9, and rpr and hid, respectively. The relative simplicity of these. . .

BSUM(65) A number of **apoptotic** and anti-**apoptotic** genes are expressed in neurons and photoreceptors, and may be involved in retinal degeneration. These cells depend on factors such as nerve growth factor and brain derived neurotrophic factor for survival, and may undergo **apoptosis** where the factor or its receptor are mutated. Among the anti-**apoptotic** genes of interest are bcl-2, bcl-xL and mcl-1. Inducers of **apoptosis** include fas (CD95), rnyc, bax, bcl-xs, TNF receptor and the **family** of cysteine proteases that includes **interleukin** 1 .beta.-**converting** enzyme.

US PAT NO: 5,686,598 [IMAGE AVAILABLE] L8: 5 of 15
SUMMARY: BSUM(8) In . . . that active photoreceptor cell death, which is characteristic of these genetically distinct disorders, is mediated by a common induction of **apoptosis**. If true, it may be possible to treat these conditions by the administration of agents that block induction of **apoptosis** in photoreceptors, such as neurotrophic factors.

BSUM(61) The . . . proteins are associated with degeneration of specific neural cells, e.g. photoreceptors, in the retina. The disease histology is consistent with **apoptosis** of affected cells. The availability of the subject gene sequences provides a means of analyzing the biology and biochemistry of. . .

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BSUM(65)
A number of **apoptotic** and anti-**apoptotic** genes are expressed in neurons and photoreceptors, and may be involved in retinal degeneration. These cells depend on factors such as nerve growth factor and brain derived neurotrophic factor for survival, and may undergo **apoptosis** where the factor or its receptor are mutated. Among the anti-**apoptotic** genes of interest are bcl-2, bcl-xL and mcl-1. Inducers of **apoptosis** include fas (CD95), myc, bax, bcl-xs, TNF receptor and the **family** of cysteine proteases that includes **interleukin** 1 .beta.-**converting** enzyme.

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(FILE 'USPAT' ENTERED AT 10:19:12 ON 14 APR 1998)

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L1     107518 S (INTERLEUKIN OR PROINTERLEUKIN)(3A)CONVERT? OR
ICE
L2        349 S APOPTO?
L3        184 S L1 AND L2
L4          0 S CASPASE?
L5         46 S INTERLEUKIN(3A)CONVERT?
L6     113522 S HOMOLOG? OR FAMIL?
L7        360 S L1(P)L6
L8         15 S L3 AND L7
L9     2710 S MCH? OR CMH?
L10        2 S L2(P)L9
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L3 1. 5,736,576, Apr. 7, 1998, Method of treating malignant tumors with thyroxine analogues having no significant hormonal activity; Ernest Kun, et al., 514/570, 568; 560/59, 60, 61, 62 [IMAGE AVAILABLE]

2. 5,736,529, Apr. 7, 1998, Use of NAD-related compounds in the treatment of neural trauma to inhibit neurodegeneration; James David Adams, et al., 514/46, 45 [IMAGE AVAILABLE]

3. 5,736,576, Apr. 7, 1998, Method of treating malignant tumors with thyroxine analogues having no significant hormonal activity; Ernest Kun, et al., 514/570, 568; 560/59, 60, 61, 62 [IMAGE AVAILABLE]

4. 5,736,529, Apr. 7, 1998, Use of NAD-related compounds in the treatment of neural trauma to inhibit neurodegeneration; James David Adams, et al., 514/46, 45 [IMAGE AVAILABLE]

5. 5,734,040, Mar. 31, 1998, Positively charged oligonucleotides as regulators of gene expression; Daniel L. Weeks, et al., 536/24.5; 435/325; 536/23.1 [IMAGE AVAILABLE]

6. 5,734,022, Mar. 31, 1998, Antibodies to a novel mammalian protein associated with uncontrolled cell division; Gary R. Pasternack, 530/387.1, 387.9, 388.1, 388.2, 388.85, 389.1 [IMAGE AVAILABLE]

7. 5,733,920, Mar. 31, 1998, Inhibitors of cyclin dependent kinases; Muzammil M. Mansuri, et al., 514/337, 254, 256, 274, 312, 320, 324, 432, 456; 544/238, 315, 318, 408; 546/153, 196, 283.1; 549/23, 401, 403 [IMAGE AVAILABLE]

8. 5,733,911, Mar. 31, 1998, Method for inducing death of neoplastic cells using piperazne derivatives; Gabriel F. Eilon, et al., 514/252 [IMAGE AVAILABLE]

9. 5,733,541, Mar. 31, 1998, Hematopoietic cells: compositions and methods; Russell S. Taichman, et al., 424/93.1, 93.7; 435/325, 347, 373, 375, 377 [IMAGE AVAILABLE]

10. 5,728,846, Mar. 17, 1998, Benzo[1,2-g]-chrom-3-ene and benzo[2,3-g]-thiochrom-3-ene derivatives; Vidyasagar Vuligonda, et al., 549/16; 546/282.7; 548/146, 183; 549/26, 43, 389, 459 [IMAGE AVAILABLE]

11. 5,726,051, Mar. 10, 1998, Transglutaminase gene; Bassam M. Fraij, et al., 435/193; 536/23.2 [IMAGE AVAILABLE]

12. 5,726,025, Mar. 10, 1998, Assay and reagents for detecting inhibitors of ubiquitin-dependent degradation of cell cycle regulatory proteins; Marc W. Kirschner, et al., 435/7.2, 7.23, 7.7, 7.9, 15, 172.3; 436/86, 503 [IMAGE AVAILABLE]

13. 5,726,018, Mar. 10, 1998, Nucleic acid based assays to detect a novel mammalian protein associated with uncontrolled cell division; Gary R. Pasternack, 435/6; 536/23.5, 24.31 [IMAGE AVAILABLE]

14. 5,723,666, Mar. 3, 1998, Oxime substituted tetrahydronaphthalene derivatives having retinoid and/or retinoid antagonist-like biological activity; Vidyasagar Vuligonda, et al., 564/253; 556/465; 560/35; 562/440; 564/257 [IMAGE AVAILABLE]

15. 5,723,620, Mar. 3, 1998, Acetylenes disubstituted with a 5 substituted tetrahydronaphthyl group and with an aryl or heteroaryl groups having retinoid-like biological activity; Vidyasagar Vuligonda, et al., 546/280.1, 280.4, 282.1, 284.4 [IMAGE AVAILABLE]

16. 5,723,589, Mar. 3, 1998, Carbohydrate conjugated bio-active compounds; Dusan Miljkovic, et al., 536/1.11, 124 [IMAGE AVAILABLE]

17. 5,723,581, Mar. 3, 1998, Murine and human box-dependent myc-interacting protein (Bin1); George C. Prendergast, et al., 530/350, 827, 828 [IMAGE AVAILABLE]

18. 5,723,580, Mar. 3, 1998, Ketomethylene group-containing aldehyde cysteine and serine protease inhibitors; Sankar Chatterjee, 530/332; 568/448 [IMAGE AVAILABLE]

19. 5,723,313, Mar. 3, 1998, ARF-p19, a novel regulator of the mammalian cell cycle; Charles J. Sherr, et al., 435/69.1; 530/350 [IMAGE AVAILABLE]

US PAT NO: 5,723,581 [IMAGE AVAILABLE] L3: 17 of 184
SUMMARY: BSUM(4) Myc . . . carcinomas, myc genes are amplified [M. D. Cole, Ann. Rev. Genet., 20:361-384 (1986)]. Paradoxically, under certain conditions myc can induce **apoptosis**, a regulated cell suicide process [D. S. Askew et al, Oncogene, 6:1915-1922 (1991); G. I. Evan et al, Cell, 69:119-128 (1992)]. However, loss or suppression of **apoptosis** is an important step in the malignant conversion of human tumors containing deregulated myc oncogenes, including, prominently, prostate carcinoma [T.. . .

BSUM(5) There . . . need in the art for compositions and methods of regulating a deregulated Myc protein and of exploiting and/or diagnosing its **apoptotic** potential.

DETDESC: DETD(2) The . . . to c-Myc, the binding appears to regulate the c-Myc and result in tumor suppression, by inhibiting cell growth and/or facilitating **apoptosis** (programmed cell death). The Bin1 gene has several other features suggesting it is a tumor suppressor gene. First, Bin1 inhibits. . .

DETD(8) Also . . . amino acid residues 378-451 of SEQ ID NO: 4). Preliminary data has indicated this domain may be useful in blocking **apoptosis**. Other useful fragments include about nucleotides 813-854 of SEQ ID NO: 3 (encoding a nuclear localization signal, amino acid residues. . .

DETD(44) Compositions . . . disease states. Also provided are compositions and methods for inhibiting Bin1 activity in order to ameliorate a condition in which **apoptosis** is activated and Bin1 plays a role. Such conditions may include degenerative conditions, e.g., neurodegenerative diseases.

DETD(100) Ten . . . labeled 2-4 hr in DMEM media lacking methionine and cysteine (Gibco) with 75-125 .mu.Ci/ml EXPRESS labeling reagent (NEN), washed with **ice**-cold phosphate-buffered saline, and extracted for 20 min on **ice** with RIPA buffer containing the protease inhibitors leupeptin, aprotinin, phenylmethylsulfonyl fluoride, and antipain [E. Harlow et al, Antibodies: A Laboratory. . .

US PAT NO: 5,723,313 [IMAGE AVAILABLE] L3: 19 of 184
DETDESC: DETD(173) Both . . . and Okayama, Mol. Cell. Biol. 7:2745-2752 (1987)). Cell supernatants containing infectious retroviral pseudotypes were harvested 24-60 hours post-transfection, pooled on **ice**, and filter (0.45.mu.) sterilized. Virus infections of exponentially growing mouse fibroblasts in 100 mm diameter culture dishes were performed at. . .

DETD(175) For analysis of ARF-p19 or InK4a-p16 expression, pelleted mammalian cells were disrupted in **ice**-cold cell lysis buffer (1.times.10.sup.7 cells/ml) for 1 hour on **ice**. Nuclei and debris were removed by centrifugation in a microfuge at 12,000 rpm for 10 rain at 4.degree. C. Supernatants. . .

DETD(183) The . . . only a minor (<0.2%) metaphase fraction compared to cells infected with the vector control (.about.3%). Nor did the cells undergo **apoptosis**, as determined by the following sensitive flow cytometric assay and by their lack of DNA fragmentation.

DETD(184)**Apoptosis** Assay

DETD(185) Trypsinized . . . suspensions were washed and suspended in 0.5 ml PBS and pipetted dropwise into 5 ml of 1% buffered paraformaldehyde on **ice** with gentle mechanical mixing. After 15 min incubation on **ice**, cells were pelleted, washed with 10 ml cold PBS, and the pellets were resuspended in 1 ml 70& ethanol pre-cooled. . . Samples stored at -20.degree. C. overnight were resuspended, divided into two equal aliquots, collected by centrifugation, and washed twice with **ice** cold PBS. Duplicate samples were resuspended in 50 .mu.l reaction mixtures containing 1.times. terminal deoxynucleotidyl transferase (TdT) buffer, CoCl.sub.2, and. . . a TdT kit by Boehringer Mannheim Corp., Indianapolis, Ill.). After 30 min incubation at 37 .degree. C., 1 ml of **ice** cold PBS was added, and recentrifuged cells were suspended in 100 .mu.l of a 1:40 dilution in PBS of anti-digenin-FITC. . . antibody and incubated for 30 minutes in the dark at room temperature. Cells were sequentially washed in 1 ml of **ice** cold PBS containing 2mm sodium azide and 0.35% bovine serum albumin (BSA), and then in 1 ml 0.1% Triton X-100. . . Jurkat T cells treated with 100 .mu.M etoposide for 6 hrs were routinely included as positive controls for cells undergoing **apoptosis**.

L5 1. 5,723,580, Mar. 3, 1998, Ketomethylene group-containing aldehyde cysteine and serine protease inhibitors; Sankar Chatterjee, 530/332; 568/448 [IMAGE AVAILABLE]

L10 1. 5,672,500, Sep. 30, 1997, **Mch2**, an **apoptotic** cysteine protease, and compositions for making and methods of using the same; Gerald Litwack, et al., 435/252.3, 320.1; 530/350; 536/23.2 [IMAGE AVAILABLE]

2. 5,583,160, Dec. 10, 1996, Methylsphingosine used to treat apoptosis; Yasuyuki Igarashi, et al., 514/669, 24, 114, 559, 725 [IMAGE AVAILABLE]

US PAT NO: 5,583,160 [IMAGE AVAILABLE] L10: 2 of 2
DETDESC: DETD(16) Exogenously added sphingosine is metabolized to ceramide and sphingosine-1-phosphate, and a progressive conversion from sphingosine to sphingomyelin or ceramide monohexoside (**CMH**) is observed [52]. Although ceramide, S-1-P and **CMH** failed to cause DNA fragmentation in HL-60 cells, sphingomyelin hydrolysis and ceramide generation have been reported to be implicated in a signal transduction pathway that mediates induction of **apoptosis** by TNF-.alpha. in U-937 cells [15,16]. Ceramide has no effect on PKC activity [53].

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(FILE 'USPAT' ENTERED AT 13:38:22 ON 07 OCT 1998)
L1      111617 S (INTERLEUKIN OR PROINTERLEUKIN)(3A)CONVERT? OR
ICE
L2         544 S APOPTO?
L3         275 S L1 AND L2
L4           0 S CASPASE?
L5          63 S INTERLEUKIN(3A)CONVERT?
L6      120936 S HOMOLOG? OR FAMIL?
L7         390 S L1(P)L6
L8          27 S L3 AND L7
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L3 1. 5,817,783, Oct. 6, 1998, DR-nm23 and compositions, methods of making and methods of using the same; Bruno Calabretta, et al., 536/23.1; 435/252.3, 254.11, 254.2, 320.1; 536/23.5, 24.31 [IMAGE AVAILABLE]

2. 5,814,612, Sep. 29, 1998, Retinol derivatives and uses thereof; Jochen Buck, et al., 514/21, 725; 549/453, 512, 551, 554, 561, 563; 552/10, 11, 12; 558/430; 562/867; 564/123, 152, 153; 568/823, 824, 825 [IMAGE AVAILABLE]

3. 5,814,482, Sep. 29, 1998, Eukaryotic layered vector initiation systems; Thomas W. Dubensky, Jr., et al., 435/69.3, 320.1; 536/23.1, 24.1 [IMAGE AVAILABLE]

4. 5,814,300, Sep. 29, 1998, Gene-targeted non-human mammals deficient in the SOD-1 gene; Richard W. Scott, et al., 424/9.1 [IMAGE AVAILABLE]

5. 5,811,238, Sep. 22, 1998, Methods for generating polynucleotides having desired characteristics by iterative selection and recombination; Willem P. C. Stemmer, et al., 435/6 [IMAGE AVAILABLE]

6. 5,811,097, Sep. 22, 1998, Blockade of T lymphocyte down-regulation associated with CTLA-4 signaling; James Patrick Allison, et al., 424/144.1, 130.1, 133.1, 135.1, 141.1, 143.1, 152.1, 154.1, 810; 514/2, 12, 885; 530/387.1, 387.3, 388.1, 388.22, 388.7 [IMAGE AVAILABLE]

7. 5,808,124, Sep. 15, 1998, O- or S-substituted dihydronaphthalene derivatives having retinoid and/or retinoid antagonist-like biological activity; Richard L. Beard, et al., 556/419; 534/558, 560, 564; 544/224, 229, 238, 242, 335, 336; 546/14, 281.7, 282.4, 283.7, 322, 337, 339, 342, 344, 348; 548/110, 146, 201, 215, 335.1; 549/29, 71, 214, 429, 484; 556/413, 428; 558/61, 62, 426; 560/10, 56, 80, 100, 102, 107, 128, 235; 562/426, 429, 460, 466, 490, 492; 568/27, 28, 31 [IMAGE AVAILABLE]

8. 5,808,083, Sep. 15, 1998, Acetylenes disubstituted with a 5 al[redacted]yl or heteroaryl substituted dihydronaphthyl group and with an aryl or heteroaryl group having retinoid-like biological activity; Alan T. Johnson, et al., 546/348; 548/202; 549/80, 506 [IMAGE AVAILABLE]

9. 5,808,001, Sep. 15, 1998, Human * ice* homolog antibodies and compositions thereof; Scott Michael Braxton, et al., 530/387.1, 388.1 [IMAGE AVAILABLE]

10. 5,807,862, Sep. 15, 1998, Therapeutic compounds containing pyrimidinyl moieties; J. Peter Klein, et al., 514/269; 544/309, 310, 311, 312 [IMAGE AVAILABLE]

11. 5,807,861, Sep. 15, 1998, Amine substituted xanthinyl compounds; J. Peter Klein, et al., 514/263 [IMAGE AVAILABLE]

12. 5,807,708, Sep. 15, 1998, Conservin nucleic acid molecules and compositions; Dean A. Falb, et al., 435/69.1, 252.3, 254.11, 320.1, 325; 536/23.1, 23.5 [IMAGE AVAILABLE]

13. 5,804,448, Sep. 8, 1998, Method of staining cellular material and analyzing the same; Fu-sheng Wang, et al., 436/63; 382/133, 134; 435/40.5; 436/10, 17, 164, 166, 172, 174, 800 [IMAGE AVAILABLE]

14. 5,801,236, Sep. 1, 1998, Probes for MTS1 gene and polynucleotides encoding mutant MTS1 genes; Alexander Kamb, 536/24.31; 435/320.1; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]

15. 5,801,182, Sep. 1, 1998, Amine substituted compounds; J. Peter Klein, et al., 514/269, 274; 544/310, 311, 312 [IMAGE AVAILABLE]

16. 5,801,181, Sep. 1, 1998, Amino alcohol substituted cyclic compounds; John Michnick, et al., 514/263, 183, 249, 259, 274, 309, 315, 418, 425, 617, 619, 626, 668, 669 [IMAGE AVAILABLE]

17. 5,798,442, Aug. 25, 1998, Peptidyl derivatives as inhibitors of pro-* apoptotic* cysteine proteinases; Michel Gallant, et al., 530/330, 331 [IMAGE AVAILABLE]

18. 5,798,374, Aug. 25, 1998, Methods of inhibiting phosphatase activity and treatment of disorders associated therewith; Peng Cho Tang, et al., 514/369; 548/184 [IMAGE AVAILABLE]

19. 5,798,358, Aug. 25, 1998, * Apoptosis* regulating composition; Satoru Nakai, et al., 514/254 [IMAGE AVAILABLE]

20. 5,795,964, Aug. 18, 1998, Lymphotoxin-beta and lymphotoxin-beta complexes; Jeffrey Browning, et al., 530/351, 324, 350; 536/23.4, 23.5 [IMAGE AVAILABLE]

21. 5,795,885, Aug. 18, 1998, Method of inhibiting profileration of cells by administering an aminosterol compound; Michael Zasloff, et al., 514/182 [IMAGE AVAILABLE]

22. 5,795,740, Aug. 18, 1998, Pituitary differentiation factor and methods of use thereof; Micsunica Platica, et al., 435/69.1, 252.3, 320.1, 325; 530/350, 399; 536/23.5, 24.31 [IMAGE AVAILABLE]

23. 5,795,713, Aug. 18, 1998, Methods for identifying inducers and inhibitors of programmed cell death; Bernard Roizman, et al., 435/5, 15, 21 [IMAGE AVAILABLE]

24. 5,792,772, Aug. 11, 1998, Enatiomerically pure hydroxylated xanthine compounds; James A. Bianco, et al., 514/263 [IMAGE AVAILABLE]

25. 5,792,751, Aug. 11, 1998, Tranformation of cells associated with fluid spaces; Fred D. Ledley, et al., 514/44; 435/320.1 [IMAGE AVAILABLE]

26. 5,792,635, Aug. 11, 1998, Method of inhibiting the sodium/proton exchanger NHE3 and method of inhibiting growth by administering squalamine; Michael Zasloff, 435/184; 514/182; 552/521 [IMAGE AVAILABLE]
27.

27. 5,789,567, Aug. 4, 1998, Chicken infectious anemia virus vaccine; Karel A. Schat, et al., 435/325; 424/93.2, 199.1, 204.1; 435/69.3, 235.1, 320.1; 536/23.1 [IMAGE AVAILABLE]

28. 5,789,245, Aug. 4, 1998, Alphavirus structural protein expression cassettes; Thomas W. Dubensky, Jr., et al., 435/320.1, 69.1, 325; 536/23.72 [IMAGE AVAILABLE]

29. 5,789,157, Aug. 4, 1998, Systematic evolution of ligands by exponential enrichment: tissue selex; Kirk B. Jensen, et al., 435/6, 91.2; 536/25.4 [IMAGE AVAILABLE]

30. 5,788,963, Aug. 4, 1998, Isolation and/or preservation of dendritic cells for prostate cancer immunotherapy; Gerald P. Murphy, et al., 424/93.21, 185.1, 277.1 [IMAGE AVAILABLE]

31. 5,786,191, Jul. 28, 1998, Cloning and expression of complementary DNAs for multiple members of the human cytochrome P450 2C subfamily; Joyce A. Goldstein, et al., 435/189, 7.2, 69.1, 252.3, 255.1, 320.1; 514/2, 12; 536/22.1, 23.1, 23.2, 23.5 [IMAGE AVAILABLE]

32. 5,786,173, Jul. 28, 1998, MCH[redacted]MCH5, * apoptotic* protease, nucleic acids encoding and methods of use; Emad S. Alnemri, et al., 435/69.1, 70.1, 183, 219, 252.3, 320.1; 530/324, 350; 536/23.1, 23.5, 24.31 [IMAGE AVAILABLE]

33. 5,783,667, Jul. 21, 1998, * Apoptosis* specific Tp30 protein; Eugenia Wang, 530/350; 424/130.1, 184.1; 530/387.3 [IMAGE AVAILABLE]

34. 5,783,186, Jul. 21, 1998, Antibody-induced * apoptosis* ; Tsutomu Arakawa, et al., 424/143.1, 133.1, 138.1, 141.1, 142.1; 435/330, 366; 530/387.3, 387.7, 388.15, 388.2, 388.22, 388.8, 388.85, 389.7 [IMAGE AVAILABLE]

35. 5,780,676, Jul. 14, 1998, Compounds having selective activity for Retinoid X Receptors, and means for modulation of processes mediated by Retinoid X Receptors; Marcus F. Boehm, et al., 562/490; 554/220, 221; 562/501 [IMAGE AVAILABLE]

36. 5,777,117, Jul. 7, 1998, Method for preparing substituted amino alcohol compounds; J. Peter Klein, et al., 544/267, 257, 285, 286, 287, 311; 546/141, 243, 246; 548/477, 546 [IMAGE AVAILABLE]

37. 5,777,115, Jul. 7, 1998, Acetal-and ketal-substituted pyrimidine compounds; Alistair Leigh, et al., 544/242, 267 [IMAGE AVAILABLE]

38. 5,776,902, Jul. 7, 1998, Boronophenyl analogs of phospholyrosines; William W. Bachovchin, 514/18, 19; 530/330, 331; 556/7; 564/152, 153 [IMAGE AVAILABLE]

39. 5,776,762, Jul. 7, 1998, Obesity associated genes; Michael North, et al., 435/252.3, 6, 69.1, 325; 536/23.1, 23.5, 24.3, 24.31 [IMAGE AVAILABLE]

40. 5,776,718, Jul. 7, 1998, Reversible protease inhibitors; James T. Palmer, et al., 435/23; 424/70.24; 435/4, 24; 514/1, 12, 19, 208, 588, 600, 601; 530/233, 336; 544/106 [IMAGE AVAILABLE]

41. 5,776,699, Jul. 7, 1998, Method of identifying negative hormone and/or antagonist activities; Elliott S. Klein, et al., 435/7.2, 7.1, 69.1, 320.1, 325 [IMAGE AVAILABLE]

42. 5,776,679, Jul. 7, 1998, Assays for the DNA component of human telomerase; Bryant Villeponteau, et al., 435/6, 91.2, 91.21, 91.51; 536/23.1, 24.31, 24.33 [IMAGE AVAILABLE]

43. 5,773,646, Jun. 30, 1998, Meta-substituted phenylene derivatives; Nizal Chandrakumar, et al., 562/439; 548/310.7, 321.5; 560/9, 21, 34, 35, 37, 48; 562/426 [IMAGE AVAILABLE]

44. 5,773,644, Jun. 30, 1998, Cyclopropyl alkanoic acid derivatives; Barbara B. Chen, et al., 562/439; 544/246; 560/34 [IMAGE AVAILABLE]

45. 5,773,594, Jun. 30, 1998, Alkyl or aryl substituted dihydronaphthalene derivatives having retinoid and/or retinoid antagonist-like biological activity; Alan T. Johnson, et al., 534/798; 548/204; 549/79; 560/81; 562/488 [IMAGE AVAILABLE]

46. 5,773,580, Jun. 30, 1998, Human protein kinase c inhibitor homolog; Janice Au-Young, et al., 530/350 [IMAGE AVAILABLE]

47. 5,773,573, Jun. 30, 1998, HIV peptides; Rupert Holms, 530/327; 424/184.1, 188.1, 208.1; 530/324, 325, 326 [IMAGE AVAILABLE]

48. 5,773,225, Jun. 30, 1998, Screening method for the identification ofcompounds capable of abrogation HIV-1 gag-cyclophilin complex formation;Jeremy Luban, et al., 435/7.8, 4, 7.1; 530/350 [IMAGE AVAILABLE]

49. 5,770,690, Jun. 23, 1998, Bax omega protein and methods; CatherineMastroni Bitler, et al., 530/324, 329, 350 [IMAGE AVAILABLE]

50. 5,770,595, Jun. 23, 1998, Oxime substituted therapeutic compounds;J. Peter Klein, et al., 514/263; 544/271, 273 [IMAGE AVAILABLE]

51. 5,770,580, Jun. 23, 1998, Somatic gene therapy to cells associatedwith fluid spaces; Fred D. Ledley, et al., 514/44; 435/69.1, 320.1, 325[IMAGE AVAILABLE]

52. 5,770,432, Jun. 23, 1998, Obesity associated genes; Patsy Nishina,et al., 435/252.3, 6, 325; 536/23.1, 23.5, 24.31 [IMAGE AVAILABLE]

53. 5,770,430, Jun. 23, 1998, Cellular injury response element and usesthereof; Stephen B. Howell, et al., 435/325, 320.1, 348, 366; 536/23.1,24.1 [IMAGE AVAILABLE]

54. 5,770,383, Jun. 23, 1998, Tricyclic retinoids, methods for theirproduction and use; Chan Kou Hwang, et al., 435/7.1; 514/217, 290, 454,510, 569; 530/350, 369, 412; 540/586; 546/101; 549/388; 560/8; 562/405[IMAGE AVAILABLE]

55. 5,770,382, Jun. 23, 1998, Tricyclic retinoids, methods for theirproduction and use; Chan Kou Hwang, et al., 435/7.1; 514/217, 290, 454,510, 569; 530/350, 369, 412; 540/586; 546/101; 549/388; 560/8; 562/405[IMAGE AVAILABLE]

56. 5,770,378, Jun. 23, 1998, Tricyclic retinoids, methods for theirproduction and use; Chan Kou Hwang, et al., 435/7.1; 514/217, 290, 454,510, 569; 530/350, 369, 412; 540/586; 546/101; 549/388; 560/8; 562/405[IMAGE AVAILABLE]

57. 5,763,635, Jun. 9, 1998, Tetrahydronaphthalene derivativessubstituted in the 8 position with alkyhidene groups having retinoidand/or retinoid antagonist-like biological activity; VidyasagarVuligonda, et al., 556/442, 400; 560/34, 35, 45, 48; 562/439, 440, 452,455; 564/75, 163, 180 [IMAGE AVAILABLE]

58. 5,763,566, Jun. 9, 1998, Systematic evolution of ligands byexponential enrichment: tissue SELEX; Kirk B. Jensen, et al., 530/350;435/6, 91.2; 530/300; 536/25.4 [IMAGE AVAILABLE]

59. 5,763,430, Jun. 9, 1998, Method of treating a viral infection by aministering a steroid compound; Michael Zasloff, 514/169, 170, 171,172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182 [IMAGE AVAILABLE]

60. 5,763,217, Jun. 9, 1998, Method of using, process of preparing andcomposition comprising recombinant herpesvirus vectors; Max Cynader, et al., 435/69.1, 320.1, 325, 456; 536/23.1, 24.1 [IMAGE AVAILABLE]

61. 5,763,196, Jun. 9, 1998, Assays using cross-linked polypeptide fragments of .beta.-galactosidase; Michael J. Powell, et al., 435/7.6,18, 23 [IMAGE AVAILABLE]

62. 5,760,180, Jun. 2, 1998, DNA encoding precursor of* interleukin* -1.beta. * converting* enzyme-related cysteine proteinaselll (* ICE* .sub.rel -lll); Donald W. Nicholson, et al., 530/350;435/7.1; 530/351, 387.1, 387.9, 388.23; 536/23.1 [IMAGE AVAILABLE]

63. 5,760,029, Jun. 2, 1998, Spirocycle integrin inhibitors; Prabhakar Kondaji Jadhav, et al., 514/211, 212, 255, 256, 278, 326, 364, 365, 385,396, 403, 406, 409; 540/543; 544/6; 546/16, 53, 59, 63, 70, 71, 96, 98,119, 120, 122, 139, 140, 255, 256, 268.7, 269.1, 269.7, 271.1, 271.4,272.7, 275.4, 276.4, 279.1, 281.7, 295, 333, 336; 548/131, 147, 216,300.7, 357.5, 408 [IMAGE AVAILABLE]

64. 5,760,028, Jun. 2, 1998, Integrin receptor antagonists; PrabhakarKondaji Jadhav, et al., 514/211, 81, 212, 228.2, 229.2, 242, 253, 256,258; 540/545, 554, 575, 596, 597, 598, 599, 600, 601, 602, 603; 544/67,96, 98, 179, 182, 224, 238, 262, 295, 333, 337, 350, 361, 371; 546/119,244, 268.1, 271.1, 272.4; 548/122, 127, 128, 131, 134, 136, 146, 254,255, 262.2, 312.1, 361.1 [IMAGE AVAILABLE]

65. 5,759,836, Jun. 2, 1998, Osteoarthritis-associated inducable isoformof nitric oxide synthetase; Ashok R. Amin, et al., 435/189, 184 [IMAGEAVAILABLE]

66. 5,759,809, Jun. 2, 1998, Methods of expressing proteins in insectcells and methods of killing insects; Kostas Iatrou, 435/69.1 [IMAGEAVAILABLE]

67. 5,759,536, Jun. 2, 1998, Use of fas ligand to supressT-lymphocyte-mediated immune responses; Donald Bellgrau, et al.,424/93.21, 93.1, 93.2, 93.3, 93.7 [IMAGE AVAILABLE]

68. 5,756,676, May 26, 1998, Mammalian protein associated withuncontrolled cell division; Gary R. Pasternack, 530/352; 435/7.23;530/358, 387.1, 388.73, 388.8, 389.1, 828 [IMAGE AVAILABLE]

69. 5,756,673, May 26, 1998, Regulation of smooth muscle cellproliferation; Gail E. Sonenshein, et al., 530/350 [IMAGE AVAILABLE]

70. 5,756,466, May 26, 1998, Inhibitors of * interleukin* -1.beta.* converting* enzyme; Guy W. Bemis, et al., 514/18, 16, 17, 19; 530/329,330, 331 [IMAGE AVAILABLE]

71. 5,753,674, May 19, 1998, Adenosine diphosphoribose polymerasebinding nitroso aromatic compounds useful as retroviral inactivatingagents, anti-retroviral agents, anti-retroviral agents and anti-tumoragents; Ernest Kun, et al., 514/309, 456, 617; 546/141; 549/399; 564/166 [IMAGE AVAILABLE]

72. 5,753,225, May 19, 1998, Antibodies that mimic actions of neurotrophins; Douglas O. Clary, et al., 424/130.1, 141.1, 143.1, 156.1;530/387.1, 388.1, 388.22 [IMAGE AVAILABLE]

73. 5,750,575, May 12, 1998, Substituted amino alcohol compounds; J. Peter Klein, et al., 514/617, 653; 564/182, 355, 361 [IMAGE AVAILABLE]

74. 5,750,529, May 12, 1998, * Apoptosis* regulating compostion; Satoru Nakai, et al., 514/254, 265 [IMAGE AVAILABLE]

75. 5,750,376, May 12, 1998, In vitro growth and proliferation of genetically modified multipotent neural stem cells and their progeny;Samuel Weiss, et al., 435/69.52, 69.1, 325, 368, 377, 384, 392, 395, 455,456, 458, 461 [IMAGE AVAILABLE]

76. 5,750,360, May 12, 1998, Method for quantitatively measuring * apoptosis* ; Laszlo Fesus, et al., 435/23; 426/656; 435/4, 15, 24, 26, 68.1, 110, 115; 436/63, 86, 89; 530/300, 322 [IMAGE AVAILABLE]

77. 5,750,342, May 12, 1998, Nucleic acid ligands of tissue target; Andrew Stephens, et al., . 435/6, 91.2; 536/23.1, 25.4 [IMAGE AVAILABLE]

78. 5,747,660, May 5, 1998, Nucleic acid encoding prostaglandin F.sub.2.alpha. receptor regulatory protein; David J. Orlicky, 536/23.5; 435/69.1; 536/24.31 [IMAGE AVAILABLE]

79. 5,747,650, May 5, 1998, P53AS protein and antibody therefor; Molly F. Kulesz-Martin, 530/387.7, 387.1, 388.8, 389.1, 389.2 [IMAGE AVAILABLE]

80. 5,747,645, May 5, 1998, Cytoplasmic antiproteinase-2 and cytoplasmic antiproteinase-3 and coding sequences; Cindy A. Sprecher, 530/350; 435/69.2 [IMAGE AVAILABLE]

81. 5,747,542, May 5, 1998, Oxo-substituted tetrahydronaphthalene derivatives having retinold and/or retinoid antagonist-like biological activity; Vidyasagar Vuligonda, et al., 514/646, 247, 277, 365, 374, 396, 406, 436, 438, 461, 532, 617, 640; 544/224; 546/342; 548/204, 215, 335.1, 373.1; 549/77, 80, 499; 560/100; 564/180, 253, 311 [IMAGE AVAILABLE]

82. 5,747,526, May 5, 1998, Anti-HIV /Aids Chemo(C)-, immuno(I)-, or ci-therapy using tur (or related compounds) and/or NVA (or EPV); Ariel C. Hollinshead, 514/448; 436/512; 514/21; 530/350, 413, 827, 868; 549/74 [IMAGE AVAILABLE]

83. 5,747,258, May 5, 1998, Detection of halogenated precursors incorporated into DNA; Zbigniew D. Darzynkiewicz, et al., 435/6, 29; 436/124, 164, 172 [IMAGE AVAILABLE]

84. 5,747,036, May 5, 1998, Methods and compositions for detecting and treating a subset of human patients having an autoimmune disease; Michael Brenner, et al., 424/144.1, 154.1, 173.1, 178.1 [IMAGE AVAILABLE]

85. 5,744,460, Apr. 28, 1998, Combination for treatment of proliferative diseases; Marcel Muller, et al., 514/44, 34, 242, 274, 283, 322; 536/24.5 [IMAGE AVAILABLE]

86. 5,744,339, Apr. 28, 1998, Thiomethylene group-containing aldehyde cysteine and serine protease inhibitors; Sankar Chatterjee, 435/184, 219; 514/618; 564/162 [IMAGE AVAILABLE]

87. 5,744,310, Apr. 28, 1998, Bax promoter sequence and screening assays for indentifying agents that regulate bax gene expression; John C. Reed, 435/6, 69.1, 91.4, 325; 536/24.1 [IMAGE AVAILABLE]

88. 5,741,896, Apr. 21, 1998, O- or S- substituted tetrahydronaphthalene derivatives having retinoid and/or retinoid antagonist-like biological activity; Vidyasagar Vuligonda, et al., 534/880; 560/100, 110; 562/440 [IMAGE AVAILABLE]

89. 5,741,667, Apr. 21, 1998, Tumor necrosis factor receptor-associated factors; David V. Goeddel, et al., 435/69.1, 252.3, 320.1; 536/23.5 [IMAGE AVAILABLE]

90. 5,739,338, Apr. 14, 1998, N-aryl substituted tetrahydroquinolines having retinoid agonist, retinoid antagonist or retinoid inverse agonist type biological activity; Richard L. Beard, et al., 546/153, 165 [IMAGE AVAILABLE]

91. 5,739,287, Apr. 14, 1998, Biotinylated cobalamins; D. Scott Wilbur, et al., 530/367, 409; 536/26.4, 26.41; 548/303.7 [IMAGE AVAILABLE]

US PAT NO: 5,817,783 [IMAGE AVAILABLE] L3: 1 of 275
BSUM(24) The . . . higher expression levels in the blast crisis group. DR-nm23 constitutive expression in 32Dc13 cells prevented G-CSF-induced granulocyte differentiation and caused * apoptosis* of these cells. The DR-nm23-transfected 32Dc13 cells require IL-3 for their growth, manifest a block in differentiation upon IL-3 removal. . .

BSUM(25) It is now known that BCR-ABL expression prolongs the survival of CML myeloid cells by inhibiting * apoptosis* . Coexpression of BCR-ABL and of a gene like DR-nm23 might generate a cell phenotype resembling that of CML-blast crisis marrow. . .

DETD(23) Detection of * apoptosis* by DNA gel electrophoresis

DETD(26) Equal numbers of cells were washed three times with * ice* -cold PBS prior to lysis with 1 ml lysis buffer (10 mM HEPES, pH 7.5, 150 mM NaCl, 0.5% NP-40, 10% glycerol, 10 .mu.g/ml leupeptin, 25 .mu.g/ml aprotinin, 1 mM PMSF and 1 mM EDTA). Lysates were incubated on * ice* for 30 min and centrifuged at 16,000.times.g for 15 min at 4.degree. C. After three washes with lysing buffer, loading. . .

DETD(33) At . . . function for DR-nm23 during early stages of hematopoiesis. Expression of DR-nm23 in 32Dc13 cells blocks G-CSF-induced granulocytic differentiation and causes * apoptosis* .

DETD(38) Myeloperoxidase . . . days of G-CSF treatment, after which no viable cells were recovered for RNA extraction. DR-nm23-transfected cells appeared to die of * apoptosis* as revealed by morphology, and DNA gel electrophoresis evidence of a typical DNA fragmentation pattern.

US PAT NO: 5,808,001 [IMAGE AVAILABLE] L3: 9 of 275
TITLE: Human * ice* homolog antibodies and compositions thereof

ABSTRACT: The present invention provides nucleotide and amino acid sequences that identify and encode a new human * interleukin* -1 * converting* enzyme homolog (ICEY). The present invention also provides for antisense molecules to the nucleotide sequences which encode ICEY, expression vectors. . .

BSUM(2) The . . . field of molecular biology; more particularly, the prese[redacted]tion describes the nucleic acid and amino acid sequences of a novel * interleukin* -1 * converting* enzyme homolog derived from activated THP-1 cells.

BSUM(4) To understand the * interleukin* 1 * converting* enzyme (* ICE*), it is helpful to first examine the role of interleukin-1 (IL-1), its enzymatic substrate. IL-1 facilitates host natural immunity, predominantly. . .

BSUM(5) IL-1 . . . precursors of about 31 kDa in monocytes. Pre-IL-1.beta. is cleaved to an active 17 kDa form by the IL-1.beta.-converting enzyme (* ICE*) before release from activated monocytes. On the other hand, pre-IL-1.alpha. is likely cleaved to an active 17 kDa form by a calpain-like IL-1.alpha.-converting enzyme prior to release. (Carruth et al. (1991) J. Biol. Chem. 266:12162-12167). Additionally, * ICE* has been implicated in IL-1.alpha.'s release from activated monocytes but the mechanism is not understood (Li, supra).

BSUM(6) The . . . as rheumatoid arthritis, septic shock, inflammatory bowel disease, and insulin-dependent diabetes mellitus (Li, supra). Since the cleavage of pre-IL-1.beta. by * ICE* is coupled to IL-1.beta. release and to increased IL-1 activity in the bloodstream, * ICE* activity may be higher in these pathological conditions.

BSUM(7) The importance of regulating * ICE* activity to modulate the IL-1.alpha. concentration to affect the host immune response has recently been confirmed: the crmA gene product. . .

BSUM(8) * ICE* is a novel cysteine protease that is known specifically to cleave inactive IL-1.beta. precursor to its active form. (Ayala, supra).. . . hydrophobic amino acid residue, and cleaves the bond between Asp and X. However, many Asp-X bonds are not recognized by * ICE* suggesting that flanking sequences or other criteria such as accessibility are also required for recognition and cleavage. In the case of IL-1.beta., * ICE* cleaves the precursor to form active IL-1.beta. at two sequence-specific bonds: the bond between residues Asp-27 and Gly-28 and the. . .

BSUM(9) * ICE* itself is synthesized and maintained in cells as an inactive 45 kDa precursor which is processed into the active * ICE* consisting of 20- and 10-kDa subunits, p20 and p10. (Ayala, supra). Both subunits are
derived from the 45 kDa precursor. . . all these polypeptide fragments are flanked by Asp-X residues in the intact 45 kDa precursor it is possible that the * ICE* precursor is activated autocatalytically. (Ayala, supra).

BSUM(10) The three dimensional structure of * ICE* has been determined from crystallographic studies. (Walker et al. (1994) Cell 78:343-352). First, it is apparent that the active form of * ICE* is a homodimer of catalytic domains, each of which consists of p20 and p10 subunits. Second, although the active site. . .

BSUM(11) The * ICE* gene from various sources has been sequenced and possesses homology (overall 29% homology) to the product of a gene with a possible role in * apoptosis* : the Caenorhabditis elegans gene ced-3. (Yuan et al. (1993) Cell 75: 641-652). Additionally, the * ICE* gene contains a sequence region, spanning residues 166 to 287 of the human * ICE* gene, which shares a 43% homology with ced-3. It is not known whether ced-3 acts as a cysteine protease but it contains the purported catalytic residues that are located at the * ICE* active site (Cys.sub.285 and His.sub.237). The amino acid pentapeptide Glu-Ala-Cys-Arg-Gly (QACRG), containing the active site cysteine, is the longest peptide conserved among * ICE* from mice and humans and CED-3 from three different nematodes. Additionally, ced-3 contains the same four residues whose side chains. . .

BSUM(12) Inhibition with the * ICE* -specific inhibitor crmA blocks TNF- and FAS-induced * apoptosis* . Therefore, * ICE* or a homolog is believed to be involved in TNF- and FAS-induced * apoptosis* .

BSUM(13) Additionally, * ICE* possesses a degree of homology to a gene product with a possible role in embryogenesis: the mammalian gene Nedd-2/Ich-1 is expressed during embryonic brain development and is down-regulated in the adult brain. (Yuan, supra). Nedd-2, ced-3, and * ICE* gene products are about 27% homologous with the carboxy terminal of CED-3 and p10 possessing the highest degree of homology. . .

BSUM(14) To confirm * ICE* 's role in inflammation-related diseases by controlling the levels of active IL-1.beta., * ICE* -deficient knockout mice were created (Li, supra). These genetically-engineered mice were normal physiologically but lacked the ability to process precursor IL-1.beta.. . . reduced. These mice were resistant to the lethal effects of septic shock when exposed to LPS (Li, supra). Therefore, inhibiting * ICE* activity to lower the concentration of IL-1.beta. in the bloodstream may be a method of treating inflammation-related diseases. * ICE* also may help identify patients who are susceptible to these diseases.

BSUM(15) Since * ICE* shares sequence homology to ced-3 and overexpression of * ICE* appears to induce * apoptosis* , the * ICE* -deficient mice studies were important because the mice seemed normal in terms of their development. If * ICE* played a strong role in * apoptosis* during development, the * ICE* -deficient mice should have had gross abnormalities in brain, gut, lymphoid and brain tissues (Li, supra). However, * ICE* may perform functions other than IL-1.beta. precursor cleavage. * ICE* mRNA has been detected in a greater variety of tissues than IL-1.beta. mRNA has (Miura, supra).

BSUM(16) * ICE* has attracted interest as a target for novel anti-inflammatory drugs, because the cytokine which it activates, IL-1.beta., is proinflammatory and. . . bowel disease and insulin-dependent diabetes mellitus (Dinarello and Wolff (1993) N Engl J Med 328:106-13). The provision of a new * ICE* gene and polypeptide will further drug research in screening for and designing more effective and more specific inhibitors to this pro-inflammatory substance. Not surprisingly, this * ICE* homolog was found in a library of activated monocytic cells, namely, phorbol- and endotoxin-treated THP-1 cells.

BSUM(24) The subject invention provides a unique nucleotide sequence which encodes a novel human * ICE* homolog. The new gene, also known as icey, which was identified within Incyte Clone 14775, encodes ICEY polypeptide and represents. . .

BSUM(25) The . . . pathologic activity of activated monocytes or macrophages which include the steps of testing a sample or an extract thereof with * ICE* homolog-encoding cDNA, fragments or oligomers thereof. Further aspects of the invention include the antisense of icey; cloning or expression vectors. . .

DRWD(2) FIGS. 1A, 1B, and 1C display the nucleotide sequence for * ice* homolog and the predicted amino acid sequence of * ICE* homolog.

DRWD(3) FIGS. 2A and 2B show the a[redacted]cid alignment of the novel * ICE* homolog with the reference human * ICE* . Alignments shown were produced using the multisequence alignment program of DNASTAR software (DNASTAR Inc, Madison Wis.).

DRWD(4) FIG. 3 displays an analysis of * ICE* homolog alpha regions (A), beta regions (B), turn regions (T), coil regions (C), hydrophilicity plot (H), alpha amphipathic regions (AA),. . .

DETD(3) As used herein, * ICE* homolog (ICEY) refers to a homolog of human * ICE* , naturally occurring ICEY, or active fragments thereof, which are encoded by mFNAs transcribed from the cNA of FIGS. 1A and.

DETD(20) "* ICE* inhibitors", or * ICE* -like molecules that bind IL-1 but do not activate it, can be synthesized recombinantly by substituting the cysteine for other natural. . .

DETD(21) * ICE* homologs with modified specificity can be readily synthesized by substituting the non-cysteine residues of the conserved pentapeptide.Such recombinant mutants. . .

DETD(23) The present invention provides a nucleotide sequence found within Incyte clone 14775 uniquely identifying a new, human * ICE* homolog (ICEY) of the cysteine protease family which is expressed in treated THP-1 cells. Because ICEY is specifically expressed in. . .

DETD(54) Each . . . the protein sequence were used to display the results of the homology search. This method did not immediately identify an * ICE* homolog. Subsequently, the human * ICE* sequence was obtained from GenBank and compared with the sequences in the LIFESEQ.TM. database (Incyte Pharmaceuticals, Inc.). Incyte clones 14775. . .

DETD(56) The nucleotide and amino acid sequences for the entire coding region of the human * ICE* homolog, ICEY, claimed in this invention are shown in FIGS. 1A and 1B.

DETD(58) Incyte . . . and edit the sequences from clone 14775. The confirmed icey sequence was homologous to but clearly different from any known * ICE* molecule. The complete nucleotide sequence for icey was translated, and the in-frame translation is shown in FIGS. 1A and 1B.. . . the possible translations of icey. FIGS. 2A and 2B show the comparison of the ICEY amino acid sequence with human * ICE* . Amino acids in agreement are highlighted in black. The expected structure (alpha, beta, and flexible regions), as well as hydrophilicity. . .

DETD(86) The activity of purified or expressed ICEY may be tested by the methods taught in the * ICE* references cited above. One such method calls for stimulating macrophages with LPS to induce the expression of pre-IL-1.beta. and then. . .

DETD(94) In . . . a polypeptide may be gained by modeling based on the structure of homologous proteins. However, the crystal structure of one * ICE* protein is known (Walker, 1994, supra) and can be used as a starting point. In both cases, relevant structural information. . .

US PAT NO: 5,798,358 [IMAGE AVAILABLE] L3: 19 of 275
TITLE: * Apoptosis* regulating composition

ABSTRACT: An object of the invention is to provide an * apoptosis* regulating composition. According to the invention, an * apoptosis* regulating composition is provided which comprises, as an active ingredient, at least one carbostyril derivatives of general formula (1) ##STR1##. . . BSUM(1) The present invention relates to a novel * apoptosis* regulating composition.

BSUM(2) The * apoptosis* regulating composition of the invention comprises, as an active ingredient, at least one member of carbostyril derivative represented by the. . .

BSUM(4) The inventor of this invention did further research into the compound (1) and found that they have * apoptosis* regulating or modifying (suppressing or promoting) activity, including anticancer activity and cell differentiation inducing activity, among others, which can hardly. .

BSUM(6) Another type of cell death is called * apoptosis* (programmed cell death) [Kerr, J. F. R. and Wyllie, A. H., Br. J. Cancer, 265, 239 (1972)]. It is said that * apoptosis* can occur under various physiological conditions. Morphologically, * apoptosis* is characterized by loss of contact with neighboring cells, concentration of cytoplasm, endonuclease activity-associated chromatin condensation and pyknosis, and segmentation. . . unit of DNA into DNA fragments 180-200 bases in size due to endonuclease activation is further observable. Final fragments of * apoptotic* body cells are phagocytosed by neighboring cells. This is the mechanism discussed by Duvall and Wyllie [Duvall, E. and Wyllie, A. H., Immunology Today, 7 (4), 115-119 (1986); Science, 245, 301-305 (1989)]. Wyllie further reported that glucocorticoid-induced * apoptosis* of thymocytes involves intracellular endonuclease activation [Wyllie, A. H., Nature, 284, 555-556 (1986)]. Endonuclease activity causes fragmentation, to the oligonucleotide level, of DNA in cells undergoing * apoptosis* and this can be readily confirmed by agarose gel electrophoresis.

BSUM(7) * Apoptosis* can be considered as preprogrammed cell death seen in the process of development, differentiation, or turnover of tissues [Wyllie, A.. . .

BSUM(8) In . . . ionophore or an increase in cAMP level leads to promotion of that DNA fragmentation which is characteristic of the above-mentioned * apoptosis* [Wyllie, A. H. et al., J. Pathol., 142, 67-77 (1984)] and, therefore, it is supposed that the calcium ion and/or cAMP be involved in the mechanisms of * apoptosis* . As an example so far reported, there may be mentioned * apoptosis* of HL-60 cells whose differentiation is induced by retinoic acid or the calcium ionophore [Martin, S. J., et al., J.. . .

BSUM(9) Reportedly, * apoptosis* occurs not only upon physiological cell death in the process of embryogenesis and physiological death of normal cells in active. . . M., et al., J. Immunol., 140, 689-692 (1988); Gillian, B., et al., Eur. J. Immunol., 17, 689-693 (1987)]. In addition, * apoptosis* is also inducible by some antibodies, for example anti-CD3, anti-APO-I, and anti-Fas antibodies [Trauth, B. C., et al., Science, 245,. . . C. A., et al. Nature, 337, 181-184 (1989); Tadakuma, T., et al., Eur. J. Immunol., 20, 779 (1990)] and, further, * apoptosis* has been confirmed in the findings of Nakamura et al. as obtained in spontaneous regression of malignant tumor [Nakamura, Y.,. .

BSUM(10) On . . . (a protein synthesis inhibitor) and calcium ion (Ca.sup.2+) chelating agents, among others, have been reported as being capable of repressing * apoptosis* and, in addition, cyclosporin-A (an immuno suppressant), hematopoietic system cytokines [IL-3, GM-CSF (granulocyte macrophage colony stimulating factor), G-CSF (granulocyte colony stimulating factor)], IL-2, bcl-2 gene product, and the like can

reportedly repress * apoptosis* [Cohen, J. J., J. Immunol., 132, 38 (1984); [illegible], A. H., et al., J. Pathol., 142, 67 (1984); Shi, Y.,. . . Vaux, D. L., et al., Nature, 335, 1440 (1988)]. While, for cycloheximide and actinomycin D, there is a report describing * apoptosis* induction in acute leukemia cells by cycloheximide, in small intestine crypt cells by actinomycin D, and in HL-60 cells by. . . J. Immunol., 145, 1859-1867 (1990)]. On the other hand, it is reported that cycloheximide rather suppresses, and actinomycin D potentiates, * apoptosis* of the lymphocytic tumor cells which are present before X-ray radiation and are increased by X-ray radiation. Therefore, it is suggested that the kind of cells, conditions, and other mechanisms be involved in the suppression or promotion of * apoptosis* [Igarashi, T., et al., Nippon Ketsueki Gakkaishi (Acta Hematol. Jpn.), 51 (2), 144 (1988)]. At any rate, it is currently considered that the differentiation, growth and maturation of cells are closely associated with * apoptosis* and that substances capable of playing some or other part in such cell differentiation, growth or the like are associated with * apoptosis* as well.

BSUM(11) Recently, cancer treatment with anti-Apo-I antibody has been attempted as an * apoptosis* -related therapy. Among the myelodysplastic syndrome (MDS), refractory anemia (RA) and refractory anemia wiht ring sideroblast (RARS) in which pancytopenia is. . . of retinoic acid or vitamin D.sub.3, which is a differentiation inducer for hemopoietic cells, and GM-CSF or IL-3 as an * apoptosis* regulating agent which suppresses excessive * apoptosis* of platelet producing cells whereas, in RAEB (refractory anemia with excess of blasts) and RAEB-t (RAEB in transformation) in which. . . agents, which induce differentiation of blast cells into mature blood cells, and etoposide and aclarubicin are said to act as * apoptosis* regulating agents, which suppress blast cell growth (thereby promote * apoptosis*) [Shibuya, T., J. Clin. Exp, Med., 160 (5), 319-323 (1992)].

BSUM(12) Murakami . . . of self tolerance and that this is due to deficiency in ability to eliminate autoantibodies producing cells as resulting from * apoptosis* induction by self antigen-autoantibody producing cells reactions as in normal mice [Murakami, M., et al., Nature, 357, 77-80 (1992)].

BSUM(13) Watanabe-Fukunaga et al. suggest that, for MRL lpr/lpr mice, Fas moleculs relating to * apoptosis* has abnormality and the nagative relation (* apoptosis*) mechanism of autoreactive T-cells does not work properly in thymus. Consequently, autoimmune disease occur [Watanabe-Fukunaga, R., et al., Nature, 356,. . .

BSUM(14) According to Montagnier et al., * apoptotic* DNA bands are observed in T lymphocyte extracts from HIV-infected patients. This phenomenon is observed in 90% of asymptomatic HIV-infected patients and in 100% of AIDS patients and of ARC (AIDS-related complex) patients, indicating increased * apoptosis* induction in HIV-infected patients as well [Montagnier, L., et al., Sixieme Colloque des Cent Gardes, 9-17 (1991)].

BSUM(16) Clark classified spontaneous neuronal deaths into three types and identified type I as * apoptosis* since, in type I neuronal death, morphological characteristics are identical with those in * apoptosis* and since type I cell death, together with DNA fragmentation, is involved in the cell death caused by deprivation of. . .

BSUM(17) According . . . a report by Edwards et al., NGF can inhibit programmed death of sympathetic nerve cells, hence NGF can presumably control * apoptosis* [Edwards, S. N., et al., J. Neurochemistry, 57 (6), 2140-2143 (1991)].

BSUM(19) For hepatic lesion of drug resistant virus hepatitis, acceleration of * apoptosis* which is direct or through the immune system, is considered to be involved in hepatic lesion.

BSUM(20) On . . . the growth of hepatocytes to produce a hyperplastic state, and this state is normalized by falling off and necrosis, i.e. * apoptosis* , of hepatocytes [Kerr, J. F., et al., Br. J. Cancer, 26, 239-257 (1972)]. As far as the liver is concerned, * apoptosis* is observable in hepatic hyperplasia, hyperplastic tuberculation and hepatic cancer, among others [Columbano, A., et al., Lab. Invest., 52, 670-675 (1985); Columbano, A., et al., Am. J. Pathol., 116, 441-446 (1984)] while, according to Kerr et al., * apoptosis* is not accompanied by inflammation or fibroplasia [Kerr, J. F., et al., Lancet, 2, 827-828 (1979)].

BSUM(21) In . . . the reports cited above, the present inventors consider that patients with hepatitis, whether acute or chronic, may be cured when * apoptosis* is inhibited. They further consider that, in patients in the process of transition from chronic hepatitis to hepatic cirrhosis and further to hepatic cancer, * apoptosis* is in a controlled state and thus cytotoxic T cells can induce hepatocyte inflammation, followed by fibrosis, causing aggravation to hepatic cirrhosis and that, therefore, hepatitis might be suppressed and development into cirrhosis prevented when * apoptosis* is promoted.

BSUM(22) The present invention provides an * apoptosis* regulating composition comprising, as an active ingredient, an effective amount of at least one of the compounds of general formula. . .

BSUM(23) The * apoptosis* regulating composition of this invention can regulate or control * apoptosis* and, owing to this action, is effective in the medicinal field as an anticancer agent, antiretrovirus agent, and therapeutic agent. . .

BSUM(36) The diseases against which the * apoptosis* regulating composition of the present invention can be expected to be effective based on the cell differentiation inducing and other. . . cirrhosis, idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia, maysthenia gravis, Hashimoto's disease, and insulin dependent (type I) diabetes mellitus. The * apoptosis* regulating composition of the invention is also applicable to various diseases accompanied by thrombocytopenia, for example myelodysplastic syndrome, periodic thrombocytopenia,. . .

BSUM(37) The * apoptosis* regulating composition of the present invention, when administered as an anticancer composition, for instance, induces differentiation of cancer cells, subsequently promotes or inhibits * apoptosis* induction or, directly promotes or inhibits * apoptosis* induction, and thereby produces an anticancer effect. In this case, the composition of the invention, irrespective of dosage form and/or. . .

BSUM(38) When used in the treatment of thrombocytopenia, the * apoptosis* regulating composition of the invention can produce a cell differentiation induction promoting action and at the same time an * apoptosis* suppressing action in patients with MDS such as RA or RARS, hus stimulating proliferation of hemopoietic cells and causing normal. . .

BSUM(39) For use in the treatment of thrombocytopenia, the * apoptosis* regulating composition of the invention can be used in combination with one or more other known drugs such as thrombopoiesis. . .

BSUM(40) The * apoptosis* regulating composition of the invention is useful also as a therapeutic and prophylactic agent for Alzheimer's disease. In this case,. . . Alzheimer's disease or senile dementia of Alzheimer type, the composition of the [illegible]on exhibits an NGF-like action through inhibition of * apoptosis* , thus producing the above-mentioned therapeutic and prophylactic effects. Further, in that case, the composition of the invention can be used. . .

BSUM(41) The * apoptosis* regulating composition of the present invention can be used as a cirrhosis preventive agent which controls * apoptosis* in patients with drug-induced hepatitis or viral hepatitis to thereby manifest a therapeutic effect in hepatitis and prevent hepatocytes from. . .

BSUM(42) Some dosage form examples for the * apoptosis* regulating composition of the invention, and results of pharmaceutical studies on the active ingredient compounds are presented below.

DRWD(2) FIG. 1 shows DNA fragmentation results in the test of * apoptosis* regulating effect according to the pharmacological test example 1;

DETD(19)* Apoptosis* -regulating effect

DETD(26) It is, thus, clear that the expression of DNA fragments appears time-dependently on the electrophoregram. This indicates that Compound 1 promotes * apoptosis* of CMK cells.

DETD(68) The . . . of a solution of fluorescein isothiocyanate (FITC)-labeled anti-human CD11b antibody (Mol; Coulter) and the reaction was allowed to proceed on * ice* in the dark for 30 minutes. Then, cells were washed twice with PBS (phosphate-buffered saline; Nissui Pharmaceutical) containing 0.1% of. . .

DETD(104) The . . . was allowed to react at 37.degree. C. for 1 hour. The reaction was then stopped by placing the mixture in * ice* and the whole amount was spotted on a membrane filter (DE-81 Filter, Whatman) and dried. After drying, the filter was. . .

DETD(162) The forgoing results indicate that administration of Compound 1 can be expected to ameliorate autoimmune disease on the basis of its * apoptosis* -modifying action.

DETD(182) The * apoptosis* regulating composition of the invention can be effectively used as a cancer chemotherapeutic agent by virtue of its characteristic * apoptosis* regulating and cell differentiation inducing activities. The composition is also effective as a therapeutic agent for AIDS, ARC, ATL, other. . .

US PAT NO: 5,786,173 [IMAGE AVAILABLE] L3: 32 of 275
TITLE: MCH4 and MCH5, * apoptotic* protease, nucleic acids encoding and methods of use

BSUM(3) The present invention relates generally to * apoptosis* or, programed cell death, and more particularly, to novel aspartate-specific cysteine proteases which can be used to modulate * apoptosis* for the therapeutic treatment of human diseases.

BSUM(4) * Apoptosis* is a normal physiological process of cell death that plays a critical role in the regulation of tissue homeostasis by. . . is offset by a commensurate rate of cell loss due to death. It has now become clear that disturbances in * apoptosis* , also referred to as physiological cell death or programmed cell death, that prevent or delay normal cell turnover can be. . . of proliferation and the cell cycle. Like cell division, which is controlled through complex interactions between cell cycle regulatory proteins, * apoptosis* is similarly regulated under normal circumstances by the interaction of gene products that either induce or inhibit cell death.

BSUM(5) The stimuli which regulate the function of these * apoptotic* gene products include both extracellular and intracellular signals. Either the presence or the removal of a particular stimuli can be sufficient to evoke a positive or negative * apoptotic* signal. For example, physiological stimuli that prevent or inhibit * apoptosis* include, for example, growth factors, extracellular matrix, CD40 ligand, viral gene products neutral amino acids, zinc, estrogen and androgens. In contrast, stimuli which promote * apoptosis* include growth factors such as tumor necrosis factor (TNF), Fas, and transforming growth factor .beta. (TGF.beta.), neurotransmitters, growth factor withdrawal,. . . stimuli, including those of environmental and pathogenetic origins, also exist which can either induce or inhibit programmed cell death. Although * apoptosis* is mediated by diverse signals and complex interactions of cellular gene products, the results of these interactions ultimately feed into. . .

BSUM(6) Several gene products which modulate the * apoptotic* process have now been identified. Although these products can in general be separated into two basic categories, gene products from. . . which are members of the Bcl-2 family of proteins. Bcl-2, is the best characterized member of this family and inhibits * apoptosis* when overexpressed in cells. Other members of this gene family include, for example, Bax, Bak, Bcl-x.sub.L, Bcl-x.sub.S, and Bad. While some of these proteins can prevent * apoptosis* others augment * apoptosis* (e.g. Bcl-x.sub.S and Bak, respectively).

BSUM(7) A . . . shown to be required for programmed cell death in the roundworm, C. elegans. The ASCPs family of proteases includes human * ICE* (* interleukin* -1-.beta. * converting* enzyme), ICH-1.sub.L, ICH-1.sub.S, CPP32, Mch2, Mch3, ICH-2 and * ICE* .sub.rel.sup.- III. Among the common features of these gene products is that 1) they are cysteine proteases with specificity for substrate. . . proenzyme produces two polypeptide protease subunits of approximately 20 kD (p20) and 10 kD (p10) which, in the case of * ICE* , combine non-covalently to form a tetramer comprised of two p20:p10 heterodimers. Although these proteases, when expressed in cells, induce cell death, several alternative structural forms of these proteases, such as * ICE* .delta., * ICE* .epsilon., ICH-1.sub.S and Mch2.beta., actually function to inhibit * apoptosis* .

BSUM(8) In addition to the Bcl-2 and ASCP gene families which play a role in * apoptosis* in mammalian cells, it has become increasingly apparent that other gene products exist which are important in mammalian cell death. . . been identified. Further, it is ambiguous as to whether other genes exist which belong to either of the above two * apoptotic* gene families or what role they may play in the programmed cell death pathway. Finally, it is unclear what the. . . processes within the organism. For example, recently it has been suggested that cytotoxic T-lymphocytes mediate their destructive function by inducing * apoptosis* in their target cells.

BSUM(9) * Apoptosis* functions in maintaining tissue homeostasis in a range of physiological processes such as embryonic development, immune cell regulation and normal cellular turnover. Therefore, the dysfunction, or loss of regulated * apoptosis* can lead to a variety of pathological disease states. For example, the loss of * apoptosis* can lead to the pathological accumulation of self-reactive lymphocytes such as that occurring with many autoimmune diseases. Inappropriate loss of * apoptosis* can also lead to the accumulation of virally infected cells and of hyperproliferative cells such as neoplastic or tumor cells. Similarly, the inappropriate activation of * apoptosis* can also contribute to a variety of pathological disease states including, for example, acquired immunodeficiency syndrome (AIDS), neurodegenerative diseases and

ischemic injury. Treatments which are specifically designed to modulate t[redacted]optotic* pathways in these and other pathological conditions can change the natural progression of many of these diseases.

BSUM(10) Thus, there exists a need to identify new * apoptotic* genes and their gene products and for methods of modulating this process for the therapeutic treatment of human diseases. The. . .

DRWD(4) FIG. . . . known human ASCPs and the nematode Ced-3 ASCP. The active site pentapeptide QACRG/QACQG is boxed. Based on crystal structure of * ICE* , the numbered residues within the * ICE* sequence are involved in catalysis (open boxes), and binding the substrate-carboxylate of P1 Asp (open circles). The residues adjacent to. . . and large subunits of ASCPs. The Roman numbers on the right indicate the three ASCP-subfamilies; the Ced-like subfamily (I), the * ICE* -like subfamily (II) and the Nedd2/Ich-1 subfamily (III). The asterisk indicates the nonconservative Arg to Gln substitution in Mch4 and Mch5.

DRWD(8) FIG. 7 shows potential * apoptotic* protease cascades involving the activation of multiple ASCP family members.

DRWD(9) FIG. . . . Mch5B and pCMV-SPORT-.beta.gal. Following 24 hours post-transfection, cells were fixed and stained with X-gal. Percentage of blue cells that were non-* apoptotic* is shown (i.e., viable cells). Non-* apoptotic* cells were distinguished from * apoptotic* cells by their flattened, spread morphology in the microscope using phase contrast optics (as opposed to rounded, pynotic or * apoptotic* morphology).

DETD(2) This . . . Mch4 and Mch5. These proteases are members of the aspartate-specific cysteine protease (ASCP) family of proteases which includes, for example, * ICE* , ICH-1.sub.L, ICH-1.sub.S, CPP32, Mch2, Mch3, ICH-2 and * ICE* .sub.rel.sup.- III. Similar to other ASCPS, Mch4 and Mch5 are synthesized as a larger proenzyme and become active following proteolytic cleavage. . . interaction with FADD. This interaction further indicates that through these FADD-like domains, Mch4 and Mch5 function in the fas mediated * apoptotic* pathway.

DETD(3) In one embodiment, the invention is directed to nucleic acids encoding the * apoptotic* cysteine protease Mch4 or Mch5. The nucleic acids are used to produce recombinant Mch4 or Mch5 proteases, whose activity can. . . polypeptides through FADD-like domains. Such pharmaceutical compounds are useful for the treatment or prevention of diseases which are characterized by * apoptotic* cell death. Alternatively, the Mch4 or Mch5 polypeptides can be used to screen for pharmaceutical compounds which activate or act. . . FADD-like domains. Such compounds are useful for the treatment or prevention of diseases which are characterized by the loss of * apoptotic* cell death.

DETD(10) The . . . of expressed sequence tags (ESTs) under various stringencies to identify potential new sequence fragments which may have homology to the * ICE* family of cysteine proteases. As described below,
such a search identified the Mch4 and Mch5 nucleic acids of the present. . . and also resulted in the reclassification of the cell death protease family. Previously these proteases were referred to as the * ICE* -family of proteases and thus the initial search criteria was directed to "* ICE* family" of cell death proteases. However, with the identification of Mch4 and Mch5, the proteases can now be divided into three subfamilies referred to herein as the Ced-like, * ICE* -like and Nedd2/ICH-1-like subfamilies of cell death proteases (see FIG. 3B).

DETD(11) In regard to the search for potential new sequences having homology to the previously referred to * ICE* family of proteases, novel sequences identified from the search as having homology to the * ICE* family of cell death proteases are then used to design primers for attempting PCR amplification and cloning of the actual. . . cDNA. The second primer for the amplification is designed to encompass homologous regions in nucleic acid sequences that encode known * ICE* protease family members. In this specific case, the primer was directed to the GSWFI/GSWYI pentapeptide sequence that is conserved in a number of the * ICE* /Ced-3 family of proteases. The primer design should take into account the predicted strandedness of both the EST sequence primer and. . .

DETD(12) As . . . base will yield homologous sequence matches to any query nucleotide sequence, additional criteria must be used to identify the authentic * ICE* subfamily homologue from among the non-specific homology matches. * ICE* family members share the highest degree of homology in the active site and catalytically important amino acid residues. A given. . . sites, but rather, may only include a region within the protease with cryptic homology. Confirming an EST as a novel * ICE* protease involves translation of all the positive EST hits in three different reading frames and subsequent identification of conservative active. . . a full length cDNA of the putative novel protease can be obtained and 1) analyzed for overall structural homology to * ICE* family members, 2) recombinantly expressed and analyzed for cysteine protease activity, and 3) analyzed for the induction of programmed cell. . .

DETD(19) * Apoptosis* plays a significant role in numerous pathological conditions in that programed cell death is either inhibited, resulting in increased cell. . . well as viral infections such as herpesvirus, poxvirus and adenovirus also result from increased cell survival or the inhibition of * apoptosis* .

DETD(20) In contrast, * apoptotic* diseases where enhanced programed cell death is a prevalent cause generally includes, for example, degenerative disorders such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, and Cerebellar degeneration. Other diseases associated with increased * apoptosis* include, for example, myelodysplastic syndromes such as aplastic anemia and ischemic injury including myocardial infarction, stroke and reperfusion injury.

DETD(21) The . . . the invention can be used to screen for pharmaceutical compounds and macromolecules which inhibit or promote Mch4 or Mch5 mediated * apoptosis* .

DETD(23) The . . . and proliferation. Additionally, molecules which interact with Mch4 and Mch5 can additionally be used to induce Mch4 and Mch5 mediated * apoptosis* . Such molecules can include, for example, FADD and FADD or fas-activators. Administration of Mch4 or Mch5 polypeptides and functional fragments thereof will induce * apoptosis* in treated cells and eliminate those cells characterized by increased cell survival or proliferation. Administration of non-Mch4 or Mch5 polypeptides. . .

DETD(25) The Mch4 or Mch5 polypeptides are administered by conventional methods, in dosages which are sufficient to induce * apoptosis* in the cells characterized by increased cell survival or proliferation. Such dosages are known or can be easily determined by. . .

DETD(26) In contrast to the induction o[redacted]4 or Mch5 mediated * apoptosis* for the treatment of pathological conditions characterized by increased cell survival or proliferation, inhibitors of Mch4 or Mch5 can be. . .

DETD(35) Additional . . . antibiotic. Such protection ensures that if, for example, mutations arise that produce mutant forms of Mch4 or Mch5, dysfunction of * apoptosis* will not occur.

DETD(36) As . . . the invention can be used to screen for compounds which inhibit or enhance the expression of Mch4 or Mch5 mediated * apoptotic* activity. Mch4 or Mch5 mediated * apoptotic* activity includes, for example, both the protease activity of these ASCPs and/or the FADD-like domain binding activity. Such screening methods. . .

DETD(41) To identify potentially novel members of the * ICE* family of cysteine proteases, an approach combining information from the GenBank database of human expressed sequence tags (ESTs) and PCR was employed. Initially, Ced-3/* ICE* -like * apoptotic* cysteine proteases from Jurkat T-lymphocytes were enriched by amplification of a human Jurkat CDNA library using degenerate PCR primers encoding. . . GSWFI/GSWYI pentapeptides (Fernandes-Alnemri et al., Cancer Res. 55:2737-2742 (1995a)). This amino acid sequence has been found to be conserved among * ICE* family members. Briefly, a 10 .mu.l aliquot of human Jurkat .lambda. Uni-Zap.TM. XR cDNA library containing approximately 10.sup.8 pfu was. . .

DETD(42) The . . . to DNA sequencing using T3 and T7 sequencing primers (Stratagene). This amplification and screen resulted in the identification of a Ced-3/* ICE* -like partial cDNA with high homology to CPP32 and Ced- 3.

DETD(47) The identification and sequence analysis of the novel * apoptotic* proteases described herein has now revealed that both Mch4 and Mch5 belong to the Ced-3-like subfamily of ASCPs. Briefly, previously. . . three subfamilies. The Ced-3-like ASCP subfamily includes Ced-3, CPP32, Mch2, and Mch3 (SEQ ID NOS:31-41, 32-36, 27-and 22-26, respectively). The * ICE* -like ASCP subfamily includes * ICE* , TX(ICH2, ICErel-II, Mih1) and ICErelIII (SEQ ID NOS:13-15, 42-46, 47-51 and 52-56, respectively). The NEDD-like subfamily include ICH-1 and its. . .

DETD(48) Both . . . 84:299-308 (1996)) can bind one of the two FADD-like domains in either Mch4 or Mch5 for activation and recruitment to Fas-* apoptotic* pathway. Activation of Mch4 or Mch5 by FADD can, for example, lead to activation of downstream proteases such as CPP32. . .

DETD(51) Comparison . . . is highest within the small subunit region. A similar relationship was observed with other family members such as CPP32/Mch3 and * ICE* /TX. These sequence similarities indicate that Mch4 and Mch5 similarly likely interact with each other as do their related family members. . .

DETD(54) Another . . . their amino termini. The inclusion of these domains indicates that they can interact with FADD early within the fas mediated * apoptotic* pathway to regulate programmed cell death. Consequently, FADD may bind the FADD-like domains in Mch4 or Mch5 for activation and recruitment to the fas * apoptotic* pathway. This recruitment occurs because FADD-like domains are capable of both homotypic and heterotypic interactions (Bold et al., J. Biol.. . .

DETD(55) In regard to specific amino acid residues that have been implicated to play functional roles, the crystal structure of * ICE* has indicated that the amino acid residues His237, Gly238 and Cys285 are involved in catalysis, while Arg179, Gln283, Arg341 and. . .

DETD(67) The . . . GST-fusion protein was then used for further enzymatic analyses. The activity of Mch4 was measured using bacterial lysates prepared with * ICE* buffer (25 mM HEPES, 1 mM EDTA, 5 mM DTT, 0.1% CHAPS, 10% sucrose, pH 7.5) at room temperature (24.degree.-25.degree... .

DETD(72) This Example shows that the cytotoxic T cell protease essential for induction of * apoptosis* in target cells directly activates ASCP members of the Ced-3 subfamily by cleavage into the large and small protease subunits.

DETD(75) Wild . . . Two microliters of the translation reactions were incubated with purified enzymes (100-200 ng) or bacterial lysates expressing recombinant ASCPs in * ICE* -buffer, in a final volume of 10 .mu.l. The reaction was incubated at 37.degree. C. for 1-2 hours and then analyzed. . .

DETD(79) Earlier . . . cytosol after incubation at 37.degree. C. for several hours (Nicholson et al., supra (1995)). THP-1 cytosol contains high concentration of * ICE* and possibly other * ICE* homologs that might be responsible for the additional processing at Asp28. The .about.17 kDa band is a cleavage product of. . .

DETD(90) Analysis . . . of proMch4 when incubated with recombinant CPP32 or Mch3 enzymes (FIG. 6). The activity of several other ASCPs such as * ICE* , TX and Mch2, were also tested but none of these enzymes were able to efficiently process proMch4. These data indicate that Mch4 is upstream of CPP32 and Mch3 in the * apoptotic* protease cascade.

DETD(91) The . . . 84:299-308 (1996)) can bind one of the two FADD-like domains in either proMch4 or proMch5 for activation and recruitment to Fas-* apoptotic* pathway. Activation of proMch4 or proMch5 by FADD can, for example, lead to activation of downstream proteases such as CPP32. . .

DETD(92) The . . . member to activate several other family members and vice versa results in multiple protease cascades and the generation of multiple * apoptotic* pathways. Evidence for the existence of multiple * apoptotic* pathways is corroborated from studies with mice deficient in * ICE* or Bc12. For example, thymocytes from * ICE* deficient mice remain sensitive to glucocorticoid- and ionizing radiation-induced * apoptosis* , but become resistant to antiFas-induced * apoptosis* (Kuida et al., Science 267:2000-2003 (1995)). On the other hand, T-cells from bc12 deficient mice become more sensitive to glucoccorticoid- and ionizing radiation-induced * apoptosis* , but less sensitive to antiCD3-induced * apoptosis* .

DETD(93) As shown in FIG. 7, the above results indicate the existence of multiple protease cascades that can be activated by different * apoptotic* stimuli. For example, one of these cascades involves proMch4 acting upstream of CPP32, Mch2 and Mch3. Once proMch4 is activated by certain * apoptotic* stimuli, it can process and activate the proenzymes of Mch3 and CPP32 as shown above. These two ASCPs are likely responsible for PARP cleavage in * apoptosis* . Active CPP32 can in turn activate proMch2, the only ASCP that can cleave lamin. Because CPP32, Mch3 and proMch4 are poorly inhibited by CrmA (see Table I), the above cascade would not be affected in an * ICE* -knockout mice, or inhibited by the * ICE* inhibitor Crm A. Therefore, it is likely that glucocortiocoid- and radiation-induced * apoptosis* occur through this cascade.

DETD(94) In an alternative * ICE* or an * ICE* -like pathway, activation of * ICE* or an * ICE* -like ASCP like TX by an * apoptotic* stimulus or an upstream ASCP results in CPP32, Mch2 and Mch3 activation (FIG. 7). This result is because TX can activate * ICE* (Faucheu et al., The EMBO J. 14:1914-1922 (1995)) and * ICE* can activate proCPP32 (Tewari et al., Cell 81:801-809 (1995)). Furthermore, Mch5 can process proCPP32 and proTX. This * ICE* -like pathway likely operates in the Fas-* apoptotic* pathway, since * ICE* knockout or CrmA abrogate this pathway in some cell types. Also, during Fas-induced * apoptosis* an * ICE* -like activity precedes CPP32-like activity (Enari. et al., Nature 380:723-726 (1996)). Consequently, FADD likely binds to FADD-like domain in proMch5 or proMch4 for activation and recruitment to Fas-* apoptotic* pathway. This conclusion is because these domains are capable of both homotypic and heterotypic interactions. Once bound to FADD, proMch5. . .

DETD(95) The . . . and proMch5 by binding the second C-terminal FADD-like (interacting) domain. It is likely that either proMch4 or proMch5 mediates Fas * apoptosis* by interacting with FADD. However, because they have two N-terminal FADD domains, these polypeptides can be involved in other forms of * apoptosis* . For example, proMch4 or proMch5 can be repressed under normal conditions by a repressor that sits on its N-terminal FADD. . .

DETD(96) In yet another distinct * apoptotic* protease cascade an exogenous protease is used to activate multiple endogenous ASCPs. This is the granzyme B-cascade which is used. . .

DETD(99) This Example shows the expression of proMch4 and induction of * apoptosis* in cultured cells.

DETD(100) To determine if proMch4 exhibits cell death activity, the induction of early * apoptosis* in Sf9 baculovirus cells was assessed. Briefly, Sf9 cells were infected with recombinant baculoviruses encoding full length proMch4 or full. . . as a standard (Fernandes-Alnemri et al., J. Biol. Chem. 269:30761-30764 (1994)). Cells were then examined microscopically for morphological signs of * apoptosis* such as blebbing of the cytoplasmic membrane, condensation of nuclear chromatin and release of small * apoptotic* bodies. In addition the genomic DNA was examined for internucleosomal DNA cleavage.

DETD(102) For the induction of * apoptosis* in Sf9 cells by proMch4 and CPP32 cells were infected with recombinant baculoviruses AcNPV-proMch4 or AcNPV-CPP32. * Apoptosis* was measured microscopically by counting cells with the appropriate morphology (blebbing, nuclear condensation). Alternatively, internucleosomal DNA cleavage is assessed as. . .

DETD(103) Expression of full length proMch4 in Sf9 cells caused a significant percentage of the cells to undergo * apoptosis* by about 48 h postinfection which is also manifested by induction of internucleosomal DNA cleavage. These results are consistent with. . .

DETD(105) The Mch5 FADD homology domain B induces * apoptosis* .

DETD(106) To determine if the expression of the FADD-like domain B of proMch5 (Mch5B) can induce * apoptosis* , it was cloned into a mammalian expression vector and transfected into the MCF7 human breast carcinoma cell line.

DETD(107) After transfection (36 hours), the percentage of transfected cells that were * apoptotic* was counted. FIG. 8 shows that in cells transfected with the control plasmid pcDNA3 about 50% of the cells were * apoptotic* . This result is likely due to the induction of * apoptosis* by the lipofection reagent used for DNA transfection. In contrast, about 80% of the cells transfected with Mch5B were * apoptotic* (FIG. 8). Thus, heterologous expression of Mch5 FADD-like domain B induces * apoptosis* in these cells.

DETD(108) The induction of * apoptosis* by Mch5B indicates that the mechanism by which Mch5B induces * apoptosis* is similar to the way in which the homologous domain in FADD (the FADD death effector domain) induces * apoptosis* when expressed by transfection. This mechanism involves binding of the Mch5 FADD-like domain to either the proMch4 or proMch5 pro-domains, binding induces activation of the proMch4 or proMch5 proteases and induction of * apoptosis* . SYSTEM LIMITS EXCEEDED - DISPLAY ENDED

US PAT NO: 5,783,667 [IMAGE AVAILABLE] L3: 33 of 275
TITLE: * Apoptosis* specific Tp30 protein

ABSTRACT: * Apoptosis* or programmed cell death is a tightly regulated mechanism used by the body to eliminate excess cells in a given. . . destruction of too many cells, tissue degeneration can occur. Therefore being able to identify which cells are destined to undergo * apoptosis* is critical in allowing clinicians, pathologists and researchers to develop means to detect, diagnose or treat disorders wherein the natural.

BSUM(4) Cells, . . . needed. Consequently, nature has provided a suicidal process to eliminate these extra cells. This process, termed programmed cell death or * apoptosis* , involves the activation of unique genes whose functions are involved in the actual killing process of the cells themselves. However,. . .

BSUM(7) Several . . . and p53, and several growth factors and cytokines. Included in the novel class are ced3, ced4, ced9, inter-leukin converting enzyme (* ICE*), reaper, and members of the bcl2 family. The applicability of the known gene class will be less specific, and in. ..

BSUM(8) In . . . useful only to study cells that survive, since this gene functions as a survival factor to protect cells from dying. * ICE* is a mammalian analogue of ced3. Therefore, marking specific dying cell populations can only be performed at present in mammalian cells by * ICE* . So far, large quantities of high-quality antibodies to * ICE* are not available. In addition, information on * ICE* 's presence in normal and diseased tissues is also absent. Consequently, there is a real need for the identification of a. . .

DRWD(8) FIG. 7. Kinetics of the onset of * apoptosis* in mouse 3T3 fibroblasts. Mouse 3T3 cells were deprived of serum for 30 min. 2, 6, 12, 18, 24, and. . . of DNA was loaded in each lane and analyzed as described under Experimental Procedures. DNA fragmentation to an oligonucleosomal ladder (* apoptosis*) became visible by 18 h of serum deprivation in 3T3 cells and increase in intensity with time.

DETD(3) In . . . kDa form) and senescent (terminin in the 60 kDa form) fibroblasts. The inventor observed that senescent fibroblasts were resistant to * apoptosis* upon serum deprivation up to 4 weeks and before this time there was no change in the molecular weight of. . .

DETD(4) The inventor studied changes in the size of terminin protein during * apoptosis* and concluded that the modification of terminin is one of the biochemical events in the pathways leading to an * apoptotic* death. In particular, they found that during * apoptosis* induced by serum deprivation in Swiss 3T3 mouse fibroblasts there is specific proteolytic degradating Tp90 and Tp60 into Tp30, a. . . that the proteolytic product, present in the 30-kDa form (Tp30), can be used as a biochemical marker for signalling the "* apoptotic* -related" events in fibroblasts.

DETD(12) Cell . . . (Boehringer Mannheim), 2 .mu.g/ml each of pepstatin and leupeptin (Boehringer Mannheim) and subsequently scraped and incubated for 10 min on * ice* . The samples were washed at 15,000 g at .degree. C., and the detergent-insoluble fraction was washed once with 10 mM. ..

DETD(15) Factor withdrawal is known to induce * apoptosis* in many systems. To examine the expression of terminin during the induction of cell death, the inventor chose to study. . .

DETD(20) A . . . withdrawal is its highly modulated response by protein synthesis inhibitors such as cycloheximide (CHX); a decline in protein synthesis during * apoptosis* usually delays or inhibits cell death. Since Tp30 appearance seems to be an early event during induction of cell death. . .

DETD(25) Understanding . . . currently the focus of many studies. Morphological features and DNA fragmentation into oligonucleosomal fragments are the only characteristic features of * apoptosis* in most of the systems studied so far. These features might be the result of the late events in the. . .

DETD(26) The . . . used as a good cellular marker for indicating the initiation of cell death. Serum deprivation in this cell line activates * apoptosis* as shown by DNA fragmentation into oligonucleosomal-size fragmentation. The terminin protein of 30 kDa (Tp30) appears very early after serum. . . cell death by a chemical agent in the presence of serum further suggest that the process seen here resembles other * apoptotic* events such as lucocorticoid-induced cell death (Galili et al. (1984) Cancer Res. 44:4594-5601) and that Tp30 is indeed an early. . .

DETD(27) When . . . and the same molecular weight. However, when Swiss 3T3 cells were subjected to serum deprivation as described before, they underwent * apoptotic* cell death immediately and there was specific proteolytic degradation of Tp90, Tp60, and Tp63 into the 30-kDa form of the. . . a specific protease or the accessibility or susceptibility of terminin polypeptides to a general protease action during the induction of * apoptosis* in 3T3 cells. Nevertheless this proteolytic action may play a significant role in programmed cell death and may be at. . .

DETD(28) Proteolysis of lamin B and topoisomerase I and II has been shown to occur during drug-induced * apoptosis* of myeloid (Kaufmann, S. H. (1989) Cancer Res. 49, 5870-5878) and mesenchymal cells (Ucker, D. S. Obernmiller, P. S. Eckhart,. . .

DETD(29) It . . . prevented by interference with macromolecular synthesis (Landon, C., Nowicki, M., Sugawara S., and Dennert, G. (1990) Cell Immunol. 128, 412-426). * Apoptosis* in the fibroblast system studied here required protein synthesis since the process was delayed by pretreatment of the cells with. . . by CHX treatment further suggests that the presence of Tp30 is a result of specific proteolysis of terminin as an * apoptosis* -dependent event.

DETD(30) The revival experiments with fresh serum demonstrated the time-dependent fashion for the final death event occurring during * apoptosis* . A population of mouse 3T3 fibroblasts could reenter the traverse of the cell cycle after 24 h at maximum serum. . . 5365-5369). This was exemplified with another cell line, rat fibroblasts, which during factor removal could escape the first wave of * apoptosis* and start DNA synthetic activity at about 12 h of serum deprivation (Evan G. I. et al. 1992. Cell 69:119-128).. . . 24 to 48 h of serum withdrawal, the Tp90, Tp63, and/or Tp60 proteins and are not far ahead into the * apoptotic* process. The refractoriness to do so after 24 h of serum removal could well be correlated with the extensive DNA. . .

DETD(31) Tp30 . . . h during serum deprivation and treatment with cell-killing agents; (2) its decreased concentration in cells pretreated with cycloheximide undergoing delayed * apoptosis* ; and (3) its decreased amount in cells rescued from death by adding serum back to the culture. Furthermore, a high. . . occurs already at 24 h at which time point Tp30 expression is at its maximum. These results suggest that the * apoptotic* and not necrotic event in mouse 3T3 cells during serum withdrawal is being examined.

DETD(57) Attempting . . . animals or transgenic mice bearing either knock-out or overexpression phenotypes. Terminin antibody (Mab 1.2) is a powerful tool for examining * apoptotic* death status in terms of change in dying cell numbers between normal and experimentally manipulated animals. In this context the. . .

CLAIMS: The embodiments of the invention in which an exclusive property or privilege is claimed are difined as follows:

1. A terminin * apoptosis* protein having a molecular weight of about 30 kDa.

US PAT NO: 5,760,180 [IMAGE AVAILABLE] L3: 62 of 275
TITLE: DNA encoding precursor of * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III (* ICE* .sub.rel -III)

ABSTRACT: A complementary DNA (cDNA) encoding full length form of * ICE* .sub.rel -III is identified, sequenced and isolated. The cDNA is cloned into expression vectors for expression in recombinant hosts. The cDNA is useful to produce recombinant full length * ICE* .sub.rel -III. The cDNA and the recombinant * ICE* .sub.rel -III protein derived therefrom are useful in diagnostic kits, laboratory reagents and assays. The cDNA and the recombinant * ICE* .sub.rel -III protein may be used to identify compounds that affect * ICE* .sub.rel -III function, inflammation and cell * apoptosis* . * ICE* .sub.rel -III function, inflammation and cell * apoptosis* may also be modulated by * ICE* .sub.rel -III antisense or gene therapy.

BSUM(2) Interleukin-1.beta. . . . synthesized as an inactive 31 kDa precursor(pIL- 1.beta.) that is processed to its mature 17.5 kDa form (mIL-1 .beta.) by * interleukin* -1.beta. * converting* enzyme (* ICE*), a novel cysteine proteinase. * ICE* generates fully active mIL-1 .beta. by cleaving pIL-1.beta. between Asp.sub.116 and Ala.sub.117, a unique site for prohormone processing. The sequence. . .

BSUM(3) Active human * ICE* as shown by conventional HPLC and affinity purification techniques is a heterodimer consisting of a 1:1 stoichiometric complex of 19,866 Da (p20) and 10,244 Da (p10) subunits. Cloned cDNAs have revealed that * ICE* is constituitively expressed as a 45 kDa proenzyme (p45) composed of a 14 kDa prodomain, followed by p20 which contains. . . of these sites and expression in heterologous systems indicates that the generation of active enzyme is autocatalytic. Murine and rat * ICE* have also been cloned and show a high degree of sequence similarity including these structural motifs.

BSUM(4) Recently, a family of * ICE* -like genes has begun to emerge, including the nematode cell death abnormal gene (CED-3) of Caenorhabiditis elegans, Caenorhabiditis briggsae and Caenorhabiditis. . . embroyonic developmentally downregulated (NEDD-2) gene. The predicted polypeptide sequences of these genes exhibit 29% and 27% sequence identity with human * ICE* , respectively. The sequence identity of CED-3 with * ICE* is higher in the regions corresponding to the p20 and p10 subunits of mature human * ICE* . All known sequences for * ICE* and for CED-3 contain the pentapeptide sequence -Gln-Ala-Cys-Arg-Gly- surrounding the catalytic cysteine of * ICE* or its equivalent in CED-3.

BSUM(5) Both CED-3 and murine * ICE* , when expressed by transfection in fibroblast cell lines or by microinjection into neuronal cells, cause programmed cell death (* apoptosis*) to occur. The pro- * apoptotic* effects of CED-3 or * ICE* can be prevented by co-transfection with either bcl-2, a mammalian proto-oncogene which appears to function as a cell death suppressor gene, or with the cytokine response modifier A (crmA) gene product, a serpin-like inhibitor of * ICE* .

BSUM(7) A novel human thiol proteinase termed * ICE* .sub.rel -III (* interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III) has been isolated and purified. A DNA molecule encoding the full length precursor form of the * ICE* .sub.rel -III protein has been isolated, purified and the nucleotide sequence determined. The * ICE* .sub.rel -III encoding DNA has been cloned for expression in recombinant hosts. The DNA clones produce recombinant full-length * ICE* .sub.rel -III and the individual subunits of the mature form of the enzyme. Recombinant * ICE* .sub.rel -III is useful for identifying modulators of * ICE* .sub.rel -III activity and hence modifiers of pathological conditions related to the pro-inflammatory or pro-* apoptotic* effects of * ICE* .sub.rel -III. * ICE* .sub.rel -III antisense molecules are useful for therapeutically reducing or eliminating the pro-inflammatory or pro- * apoptotic* effects of * ICE* .sub.rel -III, whereas gene transplantation or gene therapy with * ICE* .sub.rel -III is useful for enhancing the pro-inflammatory or pro-* apoptotic* effects of * ICE* .sub.rel -III. These therapies are beneficial in the treatment of immune, proliferative and degenerative diseases including, but not limited to, immune. . .

DRWD(2) FIG. 1. Panels A-C Nucleotide sequence of human * ICE* .sub.rel -III (cDNA clone T17.1.1), its complementary nucleotide sequence, and deduced amino acid sequence.

DRWD(3) FIG. 2. Alignment of the human * ICE* .sub.rel -III amino acid sequence with the amino acid sequence of human * ICE* . Identical amino acids are indicated by a vertical line between the aligned sequences, whereas highly conservative amino acid differences are. . .

DRWD(4) FIG. 3. Alignment of the human * ICE* .sub.rel -III amino acid sequence with the amino acid sequence of Caenorhabiditis elegans CED-3. Identical amino acids are indicated by a. . .

DRWD(5) FIG. 4. Alignment of the human * ICE* .sub.rel -III amino acid sequence with the amino acid sequence of murine NEDD-2. Identical amino acids are indicated by a vertical. . .

DETD(2) A complementary DNA (cDNA) which encodes the full length form of * ICE* .sub.rel -III is identified, sequenced and isolated. The cDNA is cloned into expression vectors for expression in a recombinant host. The cDNA is useful to produce recombinant full length * ICE* .sub.rel -III. The cDNA and the recombinant * ICE* .sub.rel -III protein derived therefrom are useful in the production of antibodies, diagnostic kits, laboratory reagents and assays. The cDNA and the recombinant * ICE* .sub.rel -III protein may be used to identify compounds that affect * ICE* .sub.rel -III function, inflammation and cell * apoptosis* . * ICE* .sub.rel -III antisense oligonucleotides or antisense mimetics may be clinically useful for reducing the expression of * ICE* .sub.rel - III protein and thereby reducing the pro-inflammatory or pro-* apoptotic* effects of * ICE* .sub.rel -III. Similarly, the * ICE* .sub.rel -III coding equence can be used for gene therapy to introduce * ICE* .sub.rel -III into target cells thereby enhancing the pro-inflammatory or pro-* apoptotic* effects of * ICE* .sub.rel -III.

DETD(3) A variety of cells and cell lines may be suitable for use to isolate * ICE* .sub.rel -III cDNA. Selection of suitable cells may be done by screening for * ICE* .sub.rel -III activity in cell extracts or conditioned medium using conventional techniques. Cells which possess * ICE* .sub.rel -III activity in this assay may be suitable for the isolation of * ICE* .sub.rel -III cDNA.

DETD(4) A variety of procedures may be used to molecularly clone * ICE* .sub.rel -III cDNA. These methods include, but are not limited to, direct functional expression of the * ICE* .sub.rel -III gene following the construction of an * ICE* .sub.rel -III-containing cDNA library in an appropriate expression vector system. Another method is to screen an * ICE* .sub.rel -III -containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labelled oligonucleotide probe designed from the amino acid sequence of * ICE* .sub.rel -III.

DETD(5) A variety of libraries constructed from cells may be useful for isolating * ICE* .sub.rel -III-encoding DNA. Suitable libraries may be prepared from cells or cell lines which have * ICE* .sub.rel -III activity.

DETD(7) DNA encoding * ICE* .sub.rel -III may also be isolated from a suitable genomic DNA library. Construction of genomic DNA libraries can be performed by. . .

DETD(8) The cloned * ICE* .sub.rel -III cDNA may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant * ICE* .sub.rel -III.

DETD(11) A variety of mammalian expression vectors may be used to express recombinant * ICE* .sub.rel -III in mammalian cells. Commercially- available mammalian expression vectors which may be suitable for recombinant * ICE* .sub.rel -III expression, include but are not limited to, pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110),. . .

DETD(12) DNA encoding * ICE* .sub.rel -III may also be cloned into an expression vector for expression in a recombinant host cell. Recombinant host cells may. . .

DETD(13) The . . . infection, protoplast fusion, and electroporation. The expression vector-containing cells are clonally propagated and individually analyzed to determine whether they produce * ICE* .sub.rel -III protein. Identification of * ICE* .sub.rel -III expressing host cell clones may be done by several means, including but not limited to immunological reactivity with anti-* ICE* .sub.rel -III antibodies, and the presence of host cell-associated * ICE* .sub.rel -III activity.

DETD(14) Expression of * ICE* .sub.rel -III cDNA may also be performed using in vitro produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various. . .

DETD(15) To determine the * ICE* .sub.rel -III cDNA sequence(s) that yields optimal levels of enzymatic activity and/or * ICE* .sub.rel -III protein, modifed * ICE* .sub.rel -III cDNA molecules are constructed. Host cells are transformed with the cDNA molecules and the levels of * ICE* .sub.rel -III RNA and protein are measured.

DETD(16) Levels of * ICE* .sub.rel -III protein in host cells are quantitated by a variety of methods such as immunoaffmity and/or ligand affinity techniques. * ICE* .sub.rel -III -specific affinity beads or * ICE* .sub.rel -III-specific antibodies are used to isolate .sup.35 S-methionine labelled or unlabelled * ICE* .sub.rel -III protein. Labelled * ICE* .sub.rel -III protein is analyzed by SDS-PAGE. Unlabelled * ICE* .sub.rel -III protein is detected by Western blotting, ELISA or RIA assays employing * ICE* .sub.rel -III specific antibodies.

DETD(17) Following expression of * ICE* .sub.rel -III in a recombinant host cell, * ICE* .sub.rel -III protein may be recovered to provide * ICE* .sub.rel -III in active form. Several * ICE* .sub.rel -III purification procedures are available and suitable for use. Recombinant * ICE* .sub.rel -III may be purified from cell lysates or from conditioned culture media, by various combinations of, or individual application of. . .

DETD(18) In addition, recombinant * ICE* .sub.rel -III can be separated from other cellular proteins by use of an immuno-affmity column made with monoclonal or polyclonal antibodies specific for full length nascent * ICE* .sub.rel -III or polypeptide fragments of * ICE* .sub.rel -III.

DETD(21) Monospecific antibodies to * ICE* .sub.rel -III are purified from mammalian antisera containing antibodies reactive against * ICE* .sub.rel -III or are prepared as monoclonal antibodies reactive with * ICE* .sub.rel -III using standard techniques. Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for * ICE* .sub.rel -III. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated with the * ICE* .sub.rel -III, as described above. Enzyme-specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with rabbits being preferred, with an appropriate concentration of * ICE* .sub.rel - III either with or without an immune adjuvant.

DETD(22) Monoclonal antibodies (mAb) reactive with * ICE* .sub.rel -III may be prepared by conventional methods, such as by immunizing inbred mice with * ICE* .sub.rel -III. The mice are immunized with about 0.1 mg to about 10 mg, preferably about 1 mg, of * ICE* .sub.rel -III in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant. Freund's complete adjuvant. . . about 30 weeks. Immunized mice are given one or more booster immunizations of about 0.1 to about 10 mg of * ICE* .sub.rel -III in a buffer solution such as phosphate buffered saline (PBS) by the intravenous (IV) route. Lymphocytes from antibody-positive mice. . . 14, 18, and 21 and are screened for antibody produciton by an immunoassay such as solid phase immunoradioassay (SPIRA) using * ICE* .sub.rel -III as the antigen. The culture fluids are also tested in the Ouchterlony precipitation assay to determine the isotype of.

DETD(23) In vitro production of anti-* ICE* .sub.rel -III mAb is carried out by growing the hydridoma in DMEM containing about 2% fetal calf serum to obtain sufficient. . .

DETD(24) Antibody . . . passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay (RIA) techniques. Similar assays are used to detect the presence of * ICE* .sub.rel -III in body fluids or tissue and cell extracts.

DETD(25) The above described methods for producing monospecific antibodies may be utilized to produce antibodies specific for * ICE* .sub.rel -III-polypeptide fragments or full-length nascent * ICE* .sub.rel -III polypeptide.

DETD(26) * ICE* .sub.rel -III antibody affinity columns are made by adding the antibodies to a gel support, such as Affigel-10 (Biorad), a gel. . . The column is then equilibrated in phosphate buffered saline (pH 7.3) and the cell culture supernatants or cell extracts containing * ICE* .sub.rel -III or * ICE* .sub.rel -III fragments are slowly passed through the column. The column is then washed, and the protein is eluted. The purified * ICE* .sub.rel -III protein is then dialyzed against phosphate buffered saline.

DETD(27) Kits containing * ICE* .sub.rel -III cDNA, * ICE* .sub.rel -III RNA, antibodies to * ICE* .sub.rel -III or * ICE* .sub.rel -III protein may be prepared. Such kits are used to detect DNA or RNA which hybridizes to * ICE* .sub.rel -III DNA or to detect the presence of * ICE* .sub.rel -III protein or peptide fragments in a sample. Such characterization is useful for a variety of purposes including but not. . .

DETD(28) The . . . molecules, RNA molecules, recombinant proteins and antibodies of the present invention may be used to screen and measure levels of * ICE* .sub.rel -III DNA, * ICE* .sub.rel -III RNA or * ICE* .sub.rel -III protein.

DETD(29) The . . . DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of * ICE* .sub.rel -III. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant * ICE* .sub.rel -III protein or anti-* ICE* .sub.rel -III antibodies suitable for detecting * ICE* .sub.rel -III. The carrier may also contain means for detection such as labeled antigen or enzyme substrates or the like.

DETD(30) Nucleotide sequences that are complementary to the * ICE* .sub.rel -III encoding cDNA sequence can be synthesized for antisense therapy. These antisense molecules may be DNA, stable derivatives of DNA such as phosphorothioates or methylphosphonates, RNA, stable derivatives of RNA such as 2'-O-alkylRNA, or other * ICE* .sub.rel -III antisense oligonucleotide mimetics. * ICE* .sub.rel -III antisense molecules may be introduced into cells by microinjection, liposome encapsulation or by expression from vectors harbouring the antisense sequence. * ICE* .sub.rel -III antisense therapy may be particularly useful for the treatment of diseases where it is beneficial to reduce * ICE* .sub.rel -III acitivy. * ICE* .sub.rel -III gene therapy may be used to introduce * ICE* .sub.rel -III into the cells of target organs. The * ICE* .sub.rel -III gene can be ligated into viral vectors which mediate transfer of the * ICE* .sub.rel -III DNA by infection of recipient host cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, polio virus and the like. Alternatively, * ICE* .sub.rel -III DNA can be transferred into cells for gene therapy by non-viral techniques including receptor- mediated targetted DNA transfer using. . . fusion or direct microinjection. These procedures and variations of them are suitable for ex vivo as well as in vivo * ICE* .sub.rel -III gene therapy. * ICE* .sub.rel -III gene therapy may be particularly useful for the treatment of diseases where it is beneficial to elevate * ICE* .sub.rel -III activity.

DETD(31) Pharmaceutically useful compositions comprising * ICE* .sub.rel -III protein may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples. . .

DETD(32) Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose * ICE* .sub.rel -III related disorders. The effective amount may vary according to a variety of factors such as the individual's condition, weight,. . .

DETD(34) Because . . . by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to the * ICE* .sub.rel -III sequence but will be capable of hybridizing to * ICE* .sub.rel -III DNA even in the presence of DNA oligonucleotides with mismatches. The mismatched DNA oligonucleotides may still hybridize to the * ICE* .sub.rel -III DNA to permit identification and isolation of * ICE* .sub.rel -III encoding DNA.

DETD(35) DNA encoding * ICE* .sub.rel -III from a particular organism may be used to isolate and purify homologues of * ICE* .sub.rel -III from other organisms. To accomplish this, the first * ICE* .sub.rel -III DNA may be mixed with a sample containing DNA encoding homologues of * ICE* .sub.rel -III under appropriate hybridization conditions. The hybridized DNA complex may be isolated and the DNA encoding the homologous DNA may. . .

DETD(38) As used herein, a "functional derivative" of * ICE* .sub.rel -III is a compound that possesses a biological activity (either functional or structural) that is substantially similar to the biological activity of * ICE* .sub.rel - III. The term "functional derivatives" is intended to include the "fragments," "variants," "degenerate variants," "analogs" and "homologs" or to "chemical derivatives" of * ICE* .sub.rel -III. The term "fragment" is meant to refer to any polypeptide subset of * ICE* .sub.rel -III. The term "variant" is meant to refer to a molecule substantially similar in structure and function to either the entire * ICE* .sub.rel -III molecule or to a fragment thereof. A molecule is substantially similar" to * ICE* .sub.rel -III if both molecules have substantially similar structures or if both molecules possess similar biological activity. Therefore, if the two. . .

DETD(39) The term "analog" refers to a molecule substantially similar in function to either the entire * ICE* .sub.rel -III molecule or to a fragment thereof.

DETD(41) The present invention is also directed to methods for screening for compounds which modulate that expression of DNA or RNA encoding * ICE* .sub.rel -III as well as the function of * ICE* .sub.rel -III protein in vivo. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding * ICE* .sub.rel -III or the function of * ICE* .sub.rel -III protein. Compounds that modulate the expression of DNA or RNA encoding * ICE* .sub.rel -III or the function of * ICE* .sub.rel -III protein may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine. . .

DETD(44)Molecular Cloning Of * ICE* .sub.rel -III

DETD(45) A full-length cDNA clone for * ICE* .sub.rel -III was identified by replica-filter screening of a THP1 cell (acute monocytic leukemia cell line; ATCC TIB 202) cDNA library. . .

DETD(49) The complete cDNA sequence of * ICE* .sub.rel -III (clone T17.1.1) and corresponding amino acid sequence is shown in FIG. 1. The longest open reading frame of * ICE* .sub.rel -III clone TI 7.1.1 (bases 35 to 1288) encodes a 47.7 kDa polypeptide which has 51% sequence identity (65% sequence similarity) with human * interleukin* -1.beta. * converting* enzyme (FIG. 2), 24% sequence identity (49% sequence similarity) with the Caenorhabiditis elegans CED-3 polypeptide (FIG. 3) and 24% sequence. . . murine NEDD-2 polypeptide (FIG. 4). The particularly high degree of sequence conservation surrounding the catalytic cysteine residue (Cys.sub. 299 of * ICE* .sub.rel -III, Cys.sub. 285 of human * interleukin* -1.beta. * converting* enzyme, Cys358 of CED-3) as well as other structural motifs throughout the polypeptide is consistent with * ICE* .sub.rel -III being a thiol proteinase.

DETD(51)Sub-Cloning Of The * ICE* .sub.rel -III cDNA Into Expression Vectors

DETD(52) The cDNA encoding * ICE* .sub.rel -III was sub-cloned into several vectors for expression of the * ICE* .sub.rel -III protein in transfected host cells and for in vitro transcription/translation. These vectors include pBluescript II SK+ (where expression is. . . the baculovirus transfer vector pVL1393 (where expression is driven by the polyhedrin (PH) promoter) for producing recombinant baculovirus containing the * ICE* .sub.rel -III encoding DNA sequence. The predicted/actual amino acid sequence of * ICE* .sub.rel -III is shown in FIG. 1.

DETD(53) a) pBluescrit II SK+:* ICE* .sub.rel -III. The full length * ICE* .sub.rel -III cDNA clone was retrieved from lambda bacteriophage by limited Eco RI digestion and ligated into Eco RI-cut, CIP-treated pBluescript II SK+. Separate subclones were recovered in which the sense orientation of * ICE* .sub.rel -III followed either the T7 or T3 promoters.

DETD(54) b) pcDNA I/Amp:* ICE* .sub.rel-III. To facilitate directional cloning, * ICE* .sub.rel -III was excised from a purified plasmid preparation of pBluescript II SK+:* ICE* .sub.rel -III in which the * ICE* .sub.rel -III DNA sequence was downstream of the T7 promoter using Eco RV and Xba I. The resulting Eco RV, Xba I * ICE* .sub.rel -III fragment was purified and ligated into Eco RV- cut, Xba I-cut, CIP-treated pcDNA I/Amp such that the * ICE* .sub.rel -III encoding DNA was downstream of the CMV promoter.

DETD(55) c) pSZ9016-1 :* ICE* .sub.rel -III. * ICE* .sub.rel -III was excised from pBluescript II SK+:* ICE* .sub.rel -III by limited Eco RI digestion and subsequent purification of the 1.3 Kb fragment from agarose gels. The resulting Eco RI * ICE* .sub.rel -III fragment was ligated into Eco RI-cut, CIP-treated pSZ9016-1. Subclones were selected in which the sense orientation of * ICE* .sub.rel -III was downstream of the HIV LTR promoter.

DETD(56) d) pVL1393:* ICE* .sub.rel-III and pVL 1393:T7 * ICE* .sub.rel-III HA Directional cloning of the * ICE* .sub.rel -III encoding DNA into the baculovirus transfer vector pVL1393 was mediated by excising * ICE* .sub.rel -III from pcDNA I/Amp:* ICE* .sub.rel -III with Bam HI and Xba I then ligating the resulting 1.3 Kb fragment into Bam HI-cut, Xba I-cut, CIP-treated pVL1393 producing pVL1393:* ICE* .sub.rel -III. Similarly, * ICE* .sub.rel -III was epitope tagged by engineering a T7 tag at the 5' amino terminus of the * ICE* .sub.rel -III open reading frame and a FluHA epitope at the 3' carboxy terminus. The * ICE* .sub.rel -III DNA modified in this manner was ligated into the Bam HI/Xba I sites of pVL 1393 to produce pVL 1393:T7 * ICE* .sub.rel -III HA.

DETD(58)Expression Of The * ICE* .sub.rel -III Polypeptide By In Vitro Transcription/ Translation and by Transfection Into Host Cells

DETD(59) Vectors containing the * ICE* .sub.rel -II encoding DNA sequence were used to drive the translation of the * ICE* .sub.rel -III polypeptide in rabbit reticulocyte lysates, mammalian host cells, and in baculovirus infected insect cells. The experimental procedures were essentially. . .

DETD(60) a) In vitro Transcription/Translation. pBluescript III SK+:* ICE* .sub.rel -III plasmid DNA (with * ICE* .sub.rel -III in the T7 orientation) was linearized by Bam HI digestion downstream of the * ICE* .sub.rel -III insert. The linearized plasmid was purified and used as a template for run-off transcription using T7 RNA polymerase in the presence of m7G(5')ppp(5')G. The resulting capped * ICE* .sub.rel -III transcripts were purified by LiCl precipitation and used to drive the translation of * ICE* .sub.rel - III in nuclease-pretreated rabbit reticulocyte lysate in the presence of L-[.sup.35 S]methionine. The resulting translation mixtures contained radiolabelled * ICE* .sub.rel -III protein which migrated on SDS/polyacrylamide gels with an apparent molecular mass of 45.+-.2 kDa.

DETD(61) b) Expression in Mammalian Cells. The * ICE* .sub.rel -III protein was expressed in mammalian host cells following transfection with either pcDNA I/Amp:* ICE* .sub.rel -III (under control of the CMV promoter) or pSZ9016-1:* ICE* .sub.rel -III (under control of the HIV LTR promoter). In the latter case (pSZ9016-1:* ICE* .sub.rel -III), cells were co-transfected with the TAT expressing plasmid pSZ90161:TAT. For both * ICE* .sub.rel -III expression plasmids, COS-7 cells were transfected using either DEAE-dextran or lipofection with Lipofectamine (BRL).

DETD(62) c) Expression in Insect Cells. The * ICE* .sub.rel -III-containing baculovirus transfer vector pVL1393:T7 * ICE* .sub.rel -III HA was used to produce recombinant baculovirus (Autographa californica) by in vivo homologous recombination. Epitope tagged * ICE* .sub.rel -III was then expressed in Sf9 (Spodoptera frugiperda) insect cells grown in suspension culture following infection with the * ICE* .sub.rel -III-containing recombinant baculovirus.

DETD(64)Cloning of * ICE* .sub.rel -III For Expression Of The * ICE* .sub.rel -III Polypeptide in Other Host Cell Systems

DETD(65) a) Cloning of * ICE* .sub.rel -III cDNA into a bacteria expression vector. Recombinant * ICE* .sub.rel -III is produced in a bacterium such as E. coli following the insertion of the optimal * ICE* .sub.rel -III cDNA sequence into expression vectors designed to direct the expression of heterologous proteins. These vectors may be constructed such that recombinant * ICE* .sub.rel -III is synthesized alone or as a fusion protein for subsequent manipulation. Similarly, expression may be controlled such that recombinant * ICE* .sub.rel -III is recovered as a soluble protein or within insoluble inclusion bodies. Vectors such as pBR322, pSKF, pUR, pATH, pGEX,. . .

DETD(66) b) Cloning of * ICE* .sub.rel -III cDNA into a yeast expression vector

DETD(67) Recombinant * ICE* .sub.rel -III is produced in a yeast such as Saccharomyces cerevisiae following the insertion of the optimal * ICE* .sub.rel -III cDNA cistron into expression vectors designed to direct the intracellular or extracellular expression of heterologous proteins. In the case of intracellular expression, vectors such as EmBLyex4 or the like are ligated to the * ICE* .sub.rel -III cistron [Rinas, U. et al., Biotechnology 8: 543-545 (1990); Horowitz B. et al., J. Biol. Chem. 265: 4189-4192 (1989)]. For extracellular expression, the * ICE* .sub.rel -III cistron is ligated into yeast expression vectors which fuse a secretion signal (a yeast or mammalian peptide) to the amino terminus of the * ICE* .sub.rel -III protein [Jacobson, M. A., Gene 85: 511-516 (1989); Riett L. and Bellon N. Biochem. 28: 2941-2949 (1989)].

DETD(68) c) Cloning of * ICE* .sub.rel -III cDNA into a viral expression vector

DETD(69) Recombinant * ICE* .sub.rel -III is produced in mammalian host cells, such as HeLa S3 cells, after infection with vaccinia virus containing the * ICE* .sub.rel - III cDNA sequence. To produce * ICE* .sub.rel - III:vaccinia virus, the * ICE* .sub.rel -III cDNA is first ligated into a transfer vector, such as pSC11, pTKgptF1s, pMJ601 or other suitable vector, then transferred to vaccinia virus by homologous recombination. After plaque purification and virus amplification, * ICE* .sub.rel -III:vaccinia virus is used to infect mammalian host cells and produce recombinant * ICE* .sub.rel -III protein.

DETD(71)Process for the Production of a * Interleukin* -1.beta. * Converting* Enzyme-Related Cysteine Proteinase Iii Polypeptide

DETD(72) Recombinant * ICE* .sub.rel -III is produced by

DETD(73) a) transforming a host cell with the DNA encoding * ICE* .sub.rel -III protein to produce a recombinant host cell;

DETD(74) b) culturing the recombinant host cell under conditions which allow the production of * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III; and

DETD(75) c) recovering the * interleukin* -1.beta. * converting* enzyme-relatedcysteine proteinase III.

DETD(76) The recombinant * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III is purified and characterized by standard methods.

DETD(78) Compounds that affect * interleukin* -1 .beta. * converting* enzyme-related cysteine proteinase III activity may be detected by a variety of methods. A method of identifying compounds that affect * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III comprises:

DETD(79) (a) mixing a test compound with a solution containing * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III to form a mixture;

DETD(80) (b) measuring * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III activity in the mixture; and

DETD(81) (c) comparing the * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase m activity in the mixture to a standard.

DETD(82) Compounds that affect * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III activity may be formulated into pharmaceutical compositions. Such pharmaceutical compositions may be

useful for treating diseases or conditions that are characterized by altered * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III activity. Examples of diseases wherein the * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III activity is increased include immune deficiency syndromes, pathogenic infections, cardiovascular and neurological injury, alopecia, aging, Parkinson's disease and Alzheimers disease. Treatment of such diseases comprises treatment with compounds that decrease the activity of * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III. Examples of diseases wherein the * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III activity is decreased include autoimmune diseases, leukemias, lymphomas and other cancers wherein the * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III activity is decreased. Treatment of such diseases comprises treatment with compounds that increase the activity of * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III activity.

DETD(84) DNA which is structurally related to DNA encoding * interleukin* - 1.beta. * converting* enzyme-related cysteine proteinase III is detected with a probe. A suitable probe may be derived from DNA having all or. . . 1, degenerate oligonucleotides derived from a portion of the amino acid sequence of FIG. 1 or an antibody directed against * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III.

DETD(86) A kit useful for the detection and characterization of DNA or RNA encoding * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III or * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III is prepared by conventional methods. The kit may contain DNA encoding * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III, recombinant * interleukin* - 1.beta. * converting* enzyme-related cysteine proteinase III, RNA corresponding to the DNA encoding * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III or antibodies to * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III. The kit may be used to characterized test samples, such as forensic samples or epidemiological samples.

DETD(88)Cloning of other * ICE* .sub.rel -III genes using human * ICE* .sub.rel-III gene

DETD(89) The cross hybridization of the DNA representing portions of the * ICE* .sub.rel -III gene to genomic DNA isolated from other organisms makes it possible to clone the homologous genes from the parent. . .

DETD(91) For example, purified nucleic acid encoding a functional * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III from such an animal may be isolated by hybridizing an appropriate sample with nucleic acid encoding * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III under low stringency conditions.

DETD(93)Use of mutagenized * ICE* .sub.rel -III

DETD(94) DNA encoding * ICE* .sub.rel -III is mutagenized using standard methods to produce an altered * ICE* .sub.rel -III gene. Host cells are transformed with the altered * ICE* .sub.rel -III to produce altered * ICE* .sub.rel -III protein. The altered * ICE* .sub.rel -III protein may be isolated, purified and used to characterize the function of * ICE* .sub.rel -III protein.

What is claimed is:

1. A recombinant * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III produced by a process comprising: a) transforming a host cell with a DNA encoding SEQ.ID.NO.: 4 to produce a recombinant host cell; b) culturing the recombinant host cell under conditions which allow the production of * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III; and c) recovering the recombinant * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III.

2. An isolated and purified * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III which is encoded by DNA having SEQ.ID.NO.: 3.

3. An isolated and purified * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III of having the amino acid sequence: ##STR1##

4. A kit comprising a reagent selected from the group consisting of isolated DNA encoding recombinant * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III having SEQ.ID.NO.:3, * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III having SEQ.ID.NO.:4.

US PAT NO: 5,756,466 [IMAGE AVAILABLE] L3: 70 of 275
TITLE: Inhibitors of * interleukin* -1.beta. * converting* enzyme

ABSTRACT: The present invention relates to novel classes of compounds which are inhibitors of * interleukin* -1.beta. * converting* enzyme. The * ICE* inhibitors of this invention are characterized by specific structural and hysicochemical features. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for inhibiting * ICE* activity and consequently, may be advantageously used as agents against interleukin-1 mediated diseases, including inflammatory diseases, autoimmune diseases and neurodegenerative diseases. This invention also relates to methods for inhibiting * ICE* activity and methods for treating interleukin-1 mediated diseases using the compounds and compositions of this invention.

BSUM(2) The present invention relates to novel classes of compounds which are inhibitors of * interleukin* -1.beta. * converting* enzyme ("* ICE* "). The * ICE* inhibitors of this invention are characterized by specific structural and physicochemical features. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for inhibiting * ICE* activity and consequently, may be advantageously used as agents against interleukin-1 ("IL-1") mediated diseases, including inflammatory diseases, autoimmune diseases and neurodegenerative diseases. This invention also relates to methods for inhibiting * ICE* activity and methods for treating interleukin-1 mediated diseases using the compounds and compositions of this invention.

BSUM(5) IL-1.beta. . . . is not processed by a signal peptidase. March, C. J., Nature, 315, pp. 641-647 (1985). Instead, pIL-1.beta. is cleaved by * interleukin* -1.beta. * converting* enzyme ("* ICE* ") between Asp-116 and Ala-117 to produce the biologically active C-terminal fragment found in human serum and synovial fluid. Sleath, P.. . . J. Biol. Chem., 265, pp. 14526-14528 (1992); A. D. Howard et al., J. Immunol., 147, pp. 2964-2969 (1991). Processing by * ICE* is also necessary for the transport of mature IL-1.beta. through the cell membrane.

BSUM(6) * ICE* is a cysteine protease localized primarily in monocytes. It converts precursor IL-1.beta. to the mature form. Black, R. A. et. . . FEBS Lett., 247, pp. 386-390 (1989); Kostura, M. J. et al., Proc. Natl. Acad. Sci. USA, 86, pp. 5227-5231 (1989). * ICE* , or its homologues, also appears to be involved in the regulation of cell death or * apoptosis* . Yuan, J. et al., Cell, 75, pp. 641-652 (1993); Miura, M. et al., Cell, 75, pp. 653-660 (1993); Nett-Fiordalisi, M. A. et al., J. Cell Biochem., 17B, p. 117 (1993). In particular, * ICE* or * ICE* homologues are thought to be associated with the regulation of * apoptosis* in neurogenerative diseases, such as Alzheimer's and Parkinson's disease. Marx, J. and M. Baringa, Science, 259, pp. 760-762 (1993); Gagliardini,. . .

BSUM(7) * ICE* has been previously described as a heterodimer composed of two subunits, p20 and p10 (20 kDa and 10 kDa molecular. . . p30 form, through an activation mechanism that is autocatalytic. Thornberry, N. A. et al., Nature, 356, pp. 768-774 (1992). The * ICE* proenzyme has been divided into several functional domains: a prodomain (p14), a p22/20 subunit, a polypeptide linker and a p10. . .

BSUM(8) Full . . . 94/00154. The p20 and p10 cDNA and amino acid sequences are also known. Thornberry et al., supra. Murine and rat * ICE* have also been sequenced and cloned. They have high amino acid and nucleic acid sequence homology to human * ICE* . Miller, D. K. et al., Ann. N.Y. Acad. Sci., 696, pp. 133-148 (1993); Molineaux, S. M. et al., Proc. Nat. Acad. Sci., 90, pp. 1809-1813 (1993). Knowledge of the primary structure of * ICE* , however, does not allow prediction of its tertiary structure. Nor does it afford an understanding of the structural, conformational and chemical interactions of * ICE* and its substrate pIL-1.beta. or other substrates or inhibitors.

BSUM(9) * ICE* inhibitors represent a class of compounds useful for the control of inflammation or * apoptosis* or both. Peptide and peptidyl inhibitors of * ICE* have been described. PCT patent applications WO 91/15577; WO 93/05071; WO 93/09135; WO 93/14777 and WO 93/16710; and European patent. . .

BSUM(10) Accordingly, the need exists for compounds that can effectively inhibit the action of * ICE* , for use as agents for preventing and treating chronic and acute forms of IL-1 mediated diseases, including various cancers, as. . .

BSUM(12) The present invention provides novel classes of compounds, and pharmaceutically acceptable derivatives thereof, that are useful as inhibitors of * ICE* . These compounds can be used alone or in combination with other therapeutic or prophylactic agents, such as antibiotics, immunomodulators or. . . IL-1. According to a preferred embodiment, the compounds of this invention are capable of binding to the active site of * ICE* and inhibiting the activity of that enzyme.

BSUM(13) It is a principal object of this invention to provide novel classes of inhibitors of * ICE* . These novel classes of * ICE* inhibitors are characterized by the following structural and physicochemical features:

BSUM(14) a) . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(15) b) . . . first and a second moderately hydrophobic moiety, said moieties each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(16) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(17) It is also an object of this invention to provide a method for identification, design or prediction of * ICE* inhibitors comprising the steps of:

BSUM(19) b) determining a low-energy conformation for binding of said compound to the active site of * ICE* ;

BSUM(20) c) . . . in said confomation to form at least two hydrogen bonds with the non-carbon backbone atoms of Arg-341 and Ser-339 of * ICE* ;

BSUM(21) d) evaluating the capability of said compound in said conformation to associate with at least two of the binding pockets of * ICE* selected from the group consisting of the P2 binding pocket, the P3 binding pocket, the P4 binding pocket and the. . .

BSUM(22) e) evaluating the capability of said compound in said conformation to interact with the P1 binding pocket of * ICE* ; and

BSUM(23) f) accepting or rejecting said candidate compound as an * ICE* inhibitor based on the determinations and evaluations carried out in the preceding steps.

BSUM(24) It is a further object of this invention to provide novel classes of * ICE* inhibitors represented by formulas: ##STR1##

BSUM(30) The term "active site" refers to any or all of the following sites in * ICE* : the substrate binding site, the site where an inhibitor binds and the site where the cleavage of substrate occurs. The. . .

BSUM(31) The . . . "S subsite", "S pocket", and the like, refer to binding subsites, or portions of the substrate binding site on the * ICE* molecule. The amino acid residues of the substrate are given designations according to their position relative to the scissile bond,. . . residues of the substrate are also labeled P1, P1', etc., by analogy with the substrate. The binding subsites of the * ICE* molecule which receive the residues labeled P1, P1', etc., are designated S1, S1', etc., or may alternately be designated "the. . .

BSUM(32) The terms "P2 binding pocket" or "S2 subsite" of the * ICE* active site are equivalent and are defined as the space surrounded by amino acid residues Pro-290, Val-338 or Trp-340.

BSUM(33) The terms "P3 binding pocket" or "S3 subsite" of the * ICE* active site are equivalent and are defined as the space surrounded by amino acid residues Pro-177, Arg-178, Thr-180, Arg-341 or. . .

BSUM(34) The terms "P4 binding pocket" or "S4 subsite" of the * ICE* active site are equivalent and are defined as the space surrounded by amino acid residues His-342, Met-345, Val-348, Arg-352, Asp-381,. . .

BSUM(35) The terms "P1 binding pocket" or "S1 subsite" of the * ICE* active site are equivalent and are defined as the space surrounded by amino acid residues Arg-179, His-237, Gln-283, or Arg-341.

BSUM(36) The terms "P' binding pocket" or "S' subsite" of the * ICE* active site are equivalent and are defined as the space surrounded by amino acid residues Phe-173, Ile-176, His-237, Gly-238, Ile-239,. . .

BSUM(45) The term "association" is used in reference to a condition of proximity between an inhibitor or portions thereof to an * ICE* molecule or portions thereof wherein the juxtaposition is energetically favored by electrostatic or van der Waals interactions.

BSUM(50) The term "scaffold" refers to a structural building block which forms the basis of an * ICE* inhibitor according to this invention. Various moieties and functional groups are intended to be appended to the scaffold. The scaffolds of this invention are thus depicted having open valences. Various scaffolds of * ICE* inhibitors according to this invention include the portions: ##STR2## In those scaffolds, the NH and CO or SO.sub.2 moieties represent. . . a second hydrogen bonding moiety, said moieties each being capable of forming a hydrogen bond with a backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-of Arg-341,. . .

BSUM(51) The . . . of a hydrogen bonding moiety which is capable of forming a hydrogen bond with the carbonyl oxygen of Arg-341 of * ICE* or the carbonyl oxygen of Ser-339 of * ICE* are excluded from substitution. These excluded hydrogen atoms include those which comprise an --NH-- group which is alpha to a. . .

BSUM(53) The . . . to a numerical measure of the effectiveness of a compound in inhibiting the activity of a target enzyme such as * ICE* . Lower values to K.sub.i reflect higher effectiveness. The K.sub.i value is a derived by fitting experimentally determined rate data to. . .

BSUM(55) The . . . difference between the free conformation energy of a compound and the bound conformation energy of that compound when bound to * ICE* . The strain energy can be determined by the following steps: Evaluate the energy of the molecule when it has the conformation necessary for binding to * ICE* . Then minimize and reevaluate the energy--this is the free conformation energy. The strain energy for binding of a potential inhibitor to * ICE* is the difference between the free conformation energy and the bound conformation energy. In a preferred embodiment, the strain energy. . .

BSUM(59) The . . . which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an anti-* ICE* active metabolite or residue thereof.

BSUM(62) The * ICE* inhibitors of this invention may contain one or more "asymmetric" carbon atoms and thus may occur as racemates and racemic. ..

BSUM(66) We have discovered that compounds possessing the following novel combination of features are surprisingly effective * ICE* inhibitors:

BSUM(67) a) . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(68) b) . . . first and a second moderately hydrophobic moiety, said moieties each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(69) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(71) Preferably, any moderately hydrophobic moiety associating with the P2 binding pocket of * ICE* does so in such a way that:

BSUM(72) a) . . . center of mass of the moderately hydrophobic moiety in the P2 binding pocket to the carbonyl oxygen of Arg-341 of * ICE* is between about 7.1 .ANG. and about 12.5 .ANG.;

BSUM(73) b) . . . center of mass of the moderately hydrophobic moiety in the P2 binding pocket to the amide nitrogen of Arg-341 of * ICE* is between about 6.0 .ANG. and about 12 .ANG.; and

BSUM(74) c) . . . center of mass of the moderately hydrophobic moiety in the P2 binding pocket to the carbonyl oxygen of Ser-339 of * ICE* is between about 3.77 .ANG. and about 9.5 .ANG..

BSUM(75) Preferably, any moderately hydrophobic moiety associating with the P3 binding pocket of * ICE* does so in such a way that:

BSUM(76) a) . . . center of mass of the moderately hydrophobic moiety in the P3 binding pocket to the carbonyl oxygen of Arg-341 of * ICE* is between about 3.9 .ANG. and about 9.5 .ANG.;

BSUM(77) b) . . . center of mass of the moderately hydrophobic moiety in the P3 binding pocket to the amide nitrogen of Arg-341 of * ICE* is between about 5.4 .ANG. and about 11 .ANG.; and

BSUM(78) c) . . . center of mass of the moderately hydrophobic moiety in the P3 binding pocket to the carbonyl oxygen of Ser-339 of * ICE* is between about 7.0 .ANG. and about 13 .ANG..

BSUM(79) Preferably, any moderately hydrophobic moiety associating with the P4 binding pocket of * ICE* does so in such a way that:

BSUM(80) a) . . . center of mass of the moderately hydrophobic moiety in the P4 binding pocket to the carbonyl oxygen of Arg-341 of * ICE* is between about 4.5 .ANG. and about 7.5 .ANG.;

BSUM(81) b) . . . center of mass of the moderately hydrophobic moiety in the P4 binding pocket to the amide nitrogen of Arg-341 of * ICE* is between about 5.5 .ANG. and about 8.5 .ANG.; and

BSUM(82) c) . . . center of mass of the moderately hydrophobic moiety in the P4 binding pocket to the carbonyl oxygen of Ser-339 of * ICE* is between about 8 .ANG. and about 11 .ANG..

BSUM(83) Preferably, any moderately hydrophobic moiety associating with the P' binding pocket of * ICE* does so in such a way that:

BSUM(84) a) . . . center of mass of the moderately hydrophobic moiety in the P' binding pocket to the carbonyl oxygen of Arg-341 of * ICE* is between about 11 .ANG. and about 16 .ANG.;

BSUM(85) b) . . . center of mass of the moderately hydrophobic moiety in the P' binding pocket to the amide nitrogen of Arg-341 of * ICE* is between about 10 .ANG. and about 15 .ANG.; and

BSUM(86) c) . . . center of mass of the moderately hydrophobic moiety in the P' binding pocket to the carbonyl oxygen of Ser-339 of * ICE* is between about 8 .ANG. and about 12 .ANG..

BSUM(88) Preferably, the * ICE* inhibitor is characterized by a neutral or favorable enthalpic contribution from the sum of all electrostatic interactions between the inhibitor and * ICE* when the inhibitor is bound thereto.

BSUM(89) Preferably, the * ICE* inhibitor further comprises less than two secondary amide bonds.

BSUM(90) Preferably, the * ICE* inhibitor further comprises less than two groups selected from the set consisting of secondary amide groups and carbamate groups.

BSUM(91) Preferably, when the inhibitor is bound to * ICE* , said moderately hydrophobic moieties separately associate with the P' binding pocket of * ICE* and the P2 binding pocket of * ICE* and the distance from the center of mass of the moderately hydrophobic moiety in the P' binding pocket to the. . .

BSUM(92) Preferably, when the inhibitor is bound to * ICE* , said inhibitor is bound to * ICE* , said moderately hydrophobic moieties separately associate with the P' binding pocket of * ICE* and the P3 binding pocket of * ICE* and the distance from the center of mass of the moderately hydrophobic moiety in the P' binding pocket to the. . .

BSUM(93) Preferably, when the inhibitor is bound to * ICE* , said inhibitor is bound to * ICE* , said moderately hydrophobic moieties separately associate with the P' binding pocket of * ICE* and the P4 binding pocket of * ICE* and the distance from the center of mass of the moderately hydrophobic moiety in the P' binding pocket to the. . .

BSUM(94) Preferably, when the inhibitor is bound to * ICE* , said moderately hydrophobic moieties separately associate with the P2 binding pocket of * ICE* and the P3 binding pocket of * ICE* and the distance from the center of mass of the moderately hydrophobic moiety in the P2 binding pocket to the. . .

BSUM(95) Preferably, when the inhibitor is bound to * ICE* , said moderately hydrophobic moieties separately associate with the P2 binding pocket of * ICE* and the P4 binding pocket of * ICE* and the distance from the center of mass of the moderately hydrophobic moiety in the P2 binding pocket to the. . .

BSUM(96) Preferably, when the inhibitor is bound to * ICE* , said moderately hydrophobic moieties separately associate with the P3 binding pocket of * ICE* and the P4 binding pocket of * ICE* and the distance from the center of mass of the moderately hydrophobic moiety in the P3 binding pocket to the. . .

BSUM(97) Preferably, when the inhibitor is bound to * ICE* , said first hydrogen bonding moiety forms a hydrogen bond with the carbonyl oxygen of Ser-339 of * ICE* and said second hydrogen bonding moiety forms a hydrogen bond with the carbonyl oxygen of Arg-341 of * ICE* and wherein the distance between said hydrogen bonding moieties is between about 5 .ANG. and about 7.5 .ANG..

BSUM(98) Preferably, when the inhibitor is bound to * ICE* , said first hydrogen bonding moiety forms a hydrogen bond with the carbonyl oxygen of Ser-339 of * ICE* and said second hydrogen bonding moiety forms a hydrogen bond with the amide --NH-- group of Arg-341 of * ICE* and wherein the distance between said moieties is between about 2.5 .ANG. and about 5 .ANG..

BSUM(99) Preferably, when the inhibitor is bound to * ICE* , said first hydrogen bonding moiety forms a hydrogen bond with the carbonyl oxygen of Arg-341 of * ICE* and said second hydrogen bonding moiety forms a hydrogen bond with the amide --NH-- group of Arg-341 of * ICE* and wherein the distance between said hydrogen bonding moieties is between about 2.5 .ANG. and about 4 .ANG..

BSUM(100) The . . . inhibitors of the present invention. These same means may be used to select a candidate compound for screening as an * ICE* inhibitor. This design or selection may begin with selection of the various moieties which fill binding pockets.

BSUM(115) Using . . . expensive experimentation to determine enzymatic inhibition activity of particular compounds. The method also is useful to facilitate rational design of * ICE* inhibitors and therapeutic and prophylactic agents against IL-1-mediated diseases. Accordingly, the present invention relates to such inhibitors.

BSUM(116) A . . . to carry out each of the above evaluations as well as the evaluations necessary in screening a candidate compound for * ICE* inhibiting activity. Generally, these techniques involve determining the location and binding proximity of a given moiety, the occupied space of. . .

BSUM(117) Different classes of active * ICE* inhibitors, according to this invention, may interact in similar ways with the various binding pockets of the * ICE* active site. The spatial arrangement of these important groups is often referred to as a pharmacophore. The concept of the. . .

BSUM(118) Different classes of * ICE* inhibitors of this invention may also use different scaffolds or core structures, but all of these cores will allow the. . . ability to match the pharmacophore, i.e., their structural identify relative to the shape and properties of the active site of * ICE* .

BSUM(119) The * ICE* inhibitors of one embodiment of this invention comprise a first and a second hydrogen bonding moiety, a first and a. . .

BSUM(121) An * ICE* inhibitor comprising:

BSUM(127) H . . . hydrogen bonding moiety, each of said moieties being capabl of forming a hydrogen bond with a different backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of.

BSUM(128) b) . . . moieties each being covalently bound to said scaffold and each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(129) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(140) An * ICE* inhibitor comprising:

BSUM(145) H . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(146) b) . . . moieties each being covalently bound to said scaffold and each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(147) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(157) An * ICE* inhibitor comprising:

BSUM(164) H . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(165)b) . . . moieties each being covalently bound to said scaffold and each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(166) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(175) An * ICE* inhibitor comprising:

BSUM(181) H . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(182) b) . . . moieties each being covalently bound to said scaffold and each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(183) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(202) An * ICE* inhibitor comprising:

BSUM(208) H . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(209) b) . . . moieties each being covalently bound to said scaffold and each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(210) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(221) An * ICE* inhibitor comprising:

BSUM(227) H . . . hydrogen bonding moiety, each of said moieties being capabl of forming a hydrogen bond with a different backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of. . .

BSUM(228) b) . . . moieties each being covalently bound to said scaffold and each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(229)c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(239) An * ICE* inhibitor comprising:

BSUM(244) H . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of * ICE* , said backbone atom being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of.

BSUM(245) b) . . . moieties each being covalently bound to said scaffold and each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(246) c) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(261) The * ICE* inhibitors of another embodiment of this invention comprise:

BSUM(262) a) . . . a central ring, wherein the distance between the centroid of said central ring and the alpha carbon of Cys-285 of * ICE* is between about 5.0 .ANG. and about 6.0 .ANG. when the inhibitor is bound to * ICE* and the distance between the centroid of said central ring and the alpha carbon of His-237 of * ICE* is between about 5.5 .ANG. and about 6.5 .ANG. when the inhibitor is bound to * ICE* ;

BSUM(263) b) . . . hydrogen bonding moiety, each of said moieties being capable of forming a hydrogen bond with a different backbone atom of * ICE* , said atoms being selected from the group consisting of the carbonyl oxygen of Arg-341, the amide --NH-- group of Arg-341,. . .

BSUM(264) c) . . . moieties each being covalently bound to said scaffold and each being capable of associating with a separate binding pocket of * ICE* when the inhibitor is bound thereto, said binding pocket being selected from the group consisting of the P2 binding pocket,. . .

BSUM(265) d) . . . being capable of forming one or more hydrogen bonds or salt bridges with residues in the P1 binding pocket of * ICE* .

BSUM(270) The * ICE* inhibitors of another embodiment of this invention are those of formulas: ##STR34## wherein: X.sub.1 is CH or N;

BSUM(401) The * ICE* inhibitors of this invention may be synthesized using conventional techniques. Advantageously, these compounds are conveniently synthesized from readily available starting. . .

BSUM(402) The compounds of this invention are among the most readily synthesized * ICE* inhibitors known. Previously described * ICE* inhibitors often contain four or more chiral centers and numerous peptide linkages. The relative ease with which the compounds of. . .

BSUM(406) The compounds of this invention are excellent ligands for * ICE* . Accordingly, these compounds are capable of targeting and inhibiting events in IL-1 mediated diseases, such as the conversion of precursor. . . neurodegenerative diseases. For example, the compounds of this invention inhibit the conversion of precursor IL-1.beta. to mature IL-1.beta. by inhibiting * ICE* . Because * ICE* is essential for the production of mature IL-1, inhibition of that enzyme effectively blocks initiation of IL-1 mediated physiological effects. . .

BSUM(408) Alternatively, . . . compositions either alone or together with other compounds of this invention in a manner consistent with the conventional utilization of * ICE* inhibitors in pharmaceutical compositions. For example, a compound of this invention may be combined with pharmaceutically acceptable adjuvants conventionally employed. . .

BSUM(409) The compounds of this invention may also be co-administered with other * ICE* inhibitors to increase the effect of therapy or prophylaxis against various IL-1-mediated diseases.

BSUM(412) When . . . to the patient. Alternatively, pharmaceutical or prophylactic compositions according to this invention may be comprised of a combination of an * ICE* inhibitor of this invention and another therapeutic or prophylactic agent.

BSUM(421) Inflammatory . . . sclerosis. And target neurodegenerative diseases include, for example, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and primary lateral sclerosis. The * ICE*
inhibitors of this invention may also be used to promote wound healing. And the * ICE* inhibitors of this invention may be used to treat infectious diseases.

BSUM(423) The compounds of this invention are also useful as commercial reagents which effectively bind to * ICE* or other cysteine proteases. As commercial reagents, the compounds of this invention, and their derivatives, may be used to block. . .

DETD(3) Step 1) Pick 2 hydrogen bonding moieties of * ICE* , here, the backbone C.dbd.O and N--H of Arg-341.

DETD(4) Step . . . is performed by using molecular mechanics techniques to minimize the scaffold fragment in the context of the active site of * ICE* . ##STR74## Step 3) Pick a hydrophobic pocket, here, S2, as next target and a hydrophobic moiety, here, benzene. Minimize the. . . minimum number of bonds consistent with a chemically reasonable structure. Minimize the entire composite molecule in the active site of * ICE* . ##STR78## Step 7) Evaluate the energy of the molecule when it has the conformation necessary for binding to * ICE* . Then minimize and reevaluate the energy--this is the free conformation energy. The strain energy for binding of the potential inhibitor to * ICE* is the difference between the free conformation energy and the bound conformation energy. The strain energy should be less than. . .

L5

1. 5,808,001, Sep. 15, 1998, Human ice homolog antibodies and compositions thereof; Scott Michael Braxton, et al., 530/387.1, 388.1 [IMAGE AVAILABLE]

2. 5,804,599, Sep. 8, 1998, Interleukin-1 production inhibiting compound; Takeo Tanaka, et al., 514/475, 616, 617, 619 [IMAGE AVAILABLE]

3. 5,798,442, Aug. 25, 1998, Peptidyl derivatives as inhibitors of pro-apoptotic cysteine proteinases; Michel Gallant, et al., 530/330, 331 [IMAGE AVAILABLE]

4. 5,798,247, Aug. 25, 1998, Organic-chemical compound with ice-inhibitory action; Hans-Peter Albrecht, et al., 435/212, 219, 226; 514/19 [IMAGE AVAILABLE]

5. 5,786,173, Jul. 28, 1998, MCH4 and MCH5, apoptotic protease, nucleic acids encoding and methods of use; Emad S. Alnemri, et al., 435/69.1, 70.1, 183, 219, 252.3, 320.1; 530/324, 350; 536/23.1, 23.5, 24.31 [IMAGE
AVAILABLE]

6. 5,776,762, Jul. 7, 1998, Obesity associated genes; Michael North, et al., 435/252.3, 6, 69.1, 325; 536/23.1, 23.5, 24.3, 24.31 [IMAGE AVAILABLE]

7. 5,776,718, Jul. 7, 1998, Reversible protease inhibitors; James T. Palmer, et al., 435/23; 424/70.24; 435/4, 24; 514/1, 12, 19, 208, 588, 600, 601; 530/233, 336; 544/106 [IMAGE AVAILABLE]

8. 5,773,236, Jun. 30, 1998, Assay for glutathiane transferase using polyhaloaryl-substituted reporter molecules; Zhenjun Diwu, et al., 435/15; 530/300; 549/223, 228 [IMAGE AVAILABLE]

9. 5,770,432, Jun. 23, 1998, Obesity associated genes; Patsy Nishina, et al., 435/252.3, 6, 325; 536/23.1, 23.5, 24.31 [IMAGE AVAILABLE]

10. 5,763,196, Jun. 9, 1998, Assays using cross-linked polypeptide fragments of .beta.-galactosidase; Michael J. Powell, et al., 435/7.6, 18, 23 [IMAGE AVAILABLE]

11. 5,760,180, Jun. 2, 1998, DNA encoding precursor of * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III (ICE.sub.rel -III); Donald W. Nicholson, et al., 530/350; 435/7.1; 530/351, 387.1, 387.9, 388.23; 536/23.1 [IMAGE AVAILABLE]

12. 5,756,466, May 26, 1998, Inhibitors of * interleukin* -1.beta. * converting* enzyme; Guy W. Bemis, et al., 514/18, 16, 17, 19; 530/329, 330, 331 [IMAGE AVAILABLE]

13. 5,747,645, May 5, 1998, Cytoplasmic antiproteinase-2 and cytoplasmic antiproteinase-3 and coding sequences; Cindy A. Sprecher, 530/350; 435/69.2 [IMAGE AVAILABLE]

14. 5,744,451, Apr. 28, 1998, N-substituted glutamic acid derivatives with * interleukin* -1 .beta. * converting* enzyme inhibitory activity; Hamish John Allen, et al., 514/18, 19; 530/330, 331 [IMAGE AVAILABLE]

15. 5,744,339, Apr. 28, 1998, Thiomethylene group-containing aldehyde cysteine and serine protease inhibitors; Sankar Chatterjee, 435/184, 219; 514/618; 564/162 [IMAGE AVAILABLE]

16. 5,739,280, Apr. 14, 1998, Para-nitroanilide peptides; Gaston O. Daumy, et al., 530/331; 435/24 [IMAGE AVAILABLE]

17. 5,739,279, Apr. 14, 1998, Peptidyl 4-amino-2,2-difluoro-3-oxo-1,6- hexanedioic acid derivatives as antiinflammatory agents; Ralph Pelton Robinson, 530/330 [IMAGE AVAILABLE]

L8
1. 5,811,238, Sep. 22, 1998, Methods for generating polynucleotides having desired characteristics by iterative selection and recombination; Willem P. C. Stemmer, et al., 435/6 [IMAGE AVAILABLE]

2. 5,808,001, Sep. 15, 1998, Human * ice* * homolog* antibodies and compositions thereof; Scott Michael Braxton, et al., 530/387.1, 388.1 [IMAGE AVAILABLE]

3. 5,798,442, Aug. 25, 1998, Peptidyl derivatives as inhibitors of pro-* apoptotic* cysteine proteinases; Michel Gallant, et al., 530/330, 331 [IMAGE AVAILABLE]

4. 5,786,173, Jul. 28, 1998, MCH4 and MCH5, * apoptotic* protease, nucleic acids encoding and methods of use; Emad S. Alnemri, et al., 435/69.1, 70.1, 183, 219, 252.3, 320.1; 530/324, 350; 536/23.1, 23.5, 24.31 [IMAGE AVAILABLE]

5. 5,783,667, Jul. 21, 1998, * Apoptosis* specific Tp30 protein; Eugenia Wang, 530/350; 424/130.1, 184.1; 530/387.3 [IMAGE AVAILABLE]

6. 5,776,762, Jul. 7, 1998, Obesity associated genes; Michael North, et al., 435/252.3, 6, 69.1, 325; 536/23.1, 23.5, 24.3, 24.31 [IMAGE AVAILABLE]

7. 5,776,718, Jul. 7, 1998, Reversible protease inhibitors; James T. Palmer, et al., 435/23; 424/70.24; 435/4, 24; 514/1, 12, 19, 208, 588, 600, 601; 530/233, 336; 544/106 [IMAGE AVAILABLE]

8. 5,770,432, Jun. 23, 1998, Obesity associated genes; Patsy Nishina, et al., 435/252.3, 6, 325; 536/23.1, 23.5, 24.31 [IMAGE AVAILABLE]

9. 5,760,180, Jun. 2, 1998, DNA encoding precursor of * interleukin* -1.beta. * converting* enzyme-related cysteine proteinase III (* ICE* .sub.rel -III); Donald W. Nicholson, et al., 530/350; 435/7.1; 530/351, 387.1, 387.9, 388.23; 536/23.1 [IMAGE AVAILABLE]

10. 5,756,466, May 26, 1998, Inhibitors of * interleukin* -1.beta. * converting* enzyme; Guy W. Bemis, et al., 514/18, 16, 17, 19; 530/329, 330, 331 [IMAGE AVAILABLE]

11. 5,747,645, May 5, 1998, Cytoplasmic antiproteinase-2 and cytoplasmic antiproteinase-3 and coding sequences; Cindy A. Sprecher, 530/350; 435/69.2 [IMAGE AVAILABLE]

12. 5,744,339, Apr. 28, 1998, Thiomethylene group-containing aldehyde cysteine and serine protease inhibitors; Sankar Chatterjee, 435/184, 219; 514/618; 564/162 [IMAGE AVAILABLE]

US PAT NO: 5,747,645 [IMAGE AVAILABLE] L8: 11 of 27
ABSTRACT: Cytoplasmic . . . proteins encoded thereby are useful in the purification of proteins and in the treatment of inflammatory diseases and diseases involving * apoptosis* .

BSUM(4) * Interleukin* -1.beta. * converting* enzyme (* ICE*) is an another example of a cysteine protease that plays an important role in inflammation. * ICE* is responsible for the activation of interleukin-1.beta., which is a critical cytokine in the inflammatory process. Serpins which inhibit * ICE* may therefore play an important role in inflammation. One such serpin is a viral protein encoded by the cowpox virus crmA gene. It is believed that xpression of crmA protein [illegible]s * ICE* and thereby blocks migration of inflammatory cells in cowpox lesions. (See Ray, C. A., et. al. (1992) Cell 69:597-604.) Isolated cellular serpins that inhibit * ICE* in a similar manner to the crmA protein can be useful in the modulation of the inflammatory response. Agents that. . .

BSUM(5) * ICE* is but one member of a * family* of serine proteases that play important roles in normal physiology and in pathophysiology. For example, another member of the * ICE* * family* , Ich-1, is involved in regulation of * apoptosis* . Furthermore, evidence is accumulating that regulation of * apoptosis* plays a role in a variety of different diseases, including cancer. Therefore, isolated serpin molecules which inhibit Ich-1 could be used to regulate * apoptosis* and treat a number of diseases.

BSUM(14) The . . . acid and protein compositions are also useful in the treatment of inflammatory diseases and in the treatment of diseases involving * apoptosis* . In addition, these compositions can be used in in vitro diagnostic procedures for these diseases.

BSUM(20) The . . . trypsin-like specificity. The biological activity of CAP-3 can be determined, for example, by its ability to inhibit proteases in the * ICE* * family* . Particular protein modifications considered minor would include substitution of amino acids of similar chemical properties, e.g., glutamic acid for aspartic. . .

BSUM(57) The . . . herein by reference. As an additional example, specificity for inhibition of other proteases of interest, such as those in the * ICE* * family* , is determined by adding CAP-2 and CAP-3 to preparations of these enzymes in vitro and monitoring changes in enzyme activity.

BSUM(58) Pharmacological . . . to those of skill in the art. For example, CAP-2 or CAP-3 may inhibit inflammation by inhibiting the activity of * ICE* or other serine proteases involved in the inflammation process. A number of in vitro and animal model systems are used. . . reference. The effect of CAP-2 or CAP-3 and antagonists or agonists thereof can be demonstrated by other methods, e..g, transfecting * Ice* , Ich-1L, or other gene which induces programmed cell death or cell degeneration (e.g., ced-3) into a cell which is also. . .

BSUM(59) The . . . be an endogenous inhibitor of specific trypsin-like serine proteases, and CAP-3 may be an endogenous inhibitor for members of the * ICE* * family* of proteases including Ich-1. Proteases with trypsin-like specificity are involved in many physiologically important processes, and * ICE* and Ich-1 play important roles in inflammation and * apoptosis* , respectively. Because of this, determination of CAP-2 or CAP-3 in biological samples can be useful in medicine.

DETD(22) The crmA protein functions as a specific inhibitor of * ICE* which represents a prototype of a larger * family* of * ICE* -like * homologs* . The * ICE* * family* of cysteine proteinases have been linked to both the negative and positive regulation of * apoptosis* . A human * homolog* of * ICE* has been identified and designated as Ich-1. In contrast to * ICE* , Ich-1-mediated effects on * apoptosis* of Rat-1 cells is only partially blocked by either microinjected or coexpressed crmA protein. These findings suggest that Ich-1 and the crmA serpin interact weakly and further suggests that Ich-1 and * ICE* have distinct but overlapping substrate specificities.

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

FILE 'REGISTRY' ENTERED AT 10:01:03 ON 08 OCT 1998

L1 21 EACRG/SQSP

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L2 25 L1

L2 ANSWER 1 OF 25 CAPLUS COPYRIGHT 1998 ACS
TI Complete genome sequence of Treponema pallidum, the syphilis spirochete PY 1998

L2 ANSWER 2 OF 25 CAPLUS COPYRIGHT 1998 ACS
TI The complete genome of the hyperthermophilic bacterium Aquifex aeolicus PY 1998

L2 ANSWER 3 OF 25 CAPLUS COPYRIGHT 1998 ACS
TI The complete genome sequence of the gram-positive bacterium Bacillus subtilis PY 1997

L2 ANSWER 4 OF 25 CAPLUS COPYRIGHT 1998 ACS
TI cDNAs for secreted proteins cloned by screening for signal sequences PY 1997

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TI Human .alpha.-N-acetylglucosaminidase, gene sequence, recombinant expression, and diagnosis and treatment of patients with .alpha.-N-acetylglucosaminidase deficiency PY 1997

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TI The structure of the gene for murine CTP:phosphocholine cytidylyltransferase, Ctpct. Relationship of exon structure to functional domains and identification of transcriptional start sites and potential upstream regulatory elements PY 1997

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TI Molecular dissection of a cosmid from a gene-rich region in 17q21 and characterization of a candidate gene for .alpha.-N- acetylglucosaminidase with two cDNA isoforms PY 1996

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TI Sequence analysis of the genome of the unicellular cyanobacterium Synechocystis sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions (supplement) PY 1996

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I Sequence analysis of the genome of the unicellular cyanobacterium Synechocystis sp. strain PCC6803. I. Sequence features in the 1 Mb region from map positions 64% to 92% of the genome PY 1995

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TI Isolation and characterization of MRF-1, a brain-derived DNA-binding protein with a capacity to regulate expression of myelin basic protein gene PY 1994

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TI The gene for murine CTP:phosphocholine cytidylyltransferase (Ctpct) is located on mouse chromosome 16 PY 1993

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TI Identification and developmental expression of a novel low molecular weight neuronal intermediate filament protein expressed in Xenopus laevis PY 1992

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TI Methanococcus voltae harbors four gene clusters potentially encoding two [nickel-iron] and two [nickel-iron-selenium] hydrogenases, each of the cofactor F420-reducing or F420-non-reducing types PY 1992

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TI Cloning and expression of rat liver CTP:phosphocholine cytidylyltransferase: an amphipathic protein that controls phosphatidylcholine synthesis PY 1990

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TI Expression of rat neurofilament proteins NF-L and NF-M in transfected non-neuronal cells PY 1989

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TI Cloning and expression of Escherichia coli of isopenicillin N synthetase genes from Streptomyces lipmanii and Aspergillus nidulans PY 1988

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TI The structure of a human neurofilament gene (NF-L): a unique exon-intron organization in the intermediate filament gene family PY 1987

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TI Cloning and developmental expression of the murine neurofilament gene family PY 1986

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L1 113 QACRG/SQSP

FILE 'CAPLUS' ENTERED AT 10:13:58 ON 08 OCT 1998

L2 80 L1

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TI Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia PY 1998

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TI Crystal structure of apopain complex with inhibitor and uses in rational drug design PY 1997

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TI Specific expression of CPP32 in sensory neurons of mouse embryos and activation of CPP32 in the apoptosis induced by a withdrawal of NGF. [Erratum to document cited in CA126:249294] PY 1997

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TI Cysteine proteinase Mch3 involved in apoptosis and cloning and expression of cDNAs encoding the subunits PY 1997

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TI Human cysteine protease CMH-1 cDNA sequence, recombinant production, gene therapy, and apoptosis inhibitor assay PY 1997

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TI Specific expression of CPP32 in sensory neurons of mouse embryos and activation of CPP32 in the apoptosis induced by a withdrawal of NGF PY 1997

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TI Induction of apoptosis and CPP32 expression by thyroid hormone in a myoblastic cell line derived from tadpole tail PY 1997

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TI Cloning and expression of a rat brain interleukin-1 .beta.-converting enzyme (ICE)-related protease (IRP) and its possible role in apoptosis of cultured cerebellar granule neurons PY 1997

L2 ANSWER 22 OF 80 CAPLUS COPYRIGHT 1998 ACS
TI Identification and mapping of Casp7, a cysteine protease resembling CPP32.beta., interleukin-1.beta. converting enzyme, and CED-3 PY 1997

21. 5,837,478, Nov. 17, 1998, Method of identifying modulators of binding between and VCAM-1; W. Michael Gallatin, et al., 435/7.24, 7.1, 7.2, 7.21, 7.8 [IMAGE AVAILABLE]

22. 5,834,228, Nov. 10, 1998, Method for identifying inhibitors for apopain based upon the crystal structure of the apopain: Ac-DEVD-CHO complex; Joseph W. Becker, et al., 435/23, 212 [IMAGE AVAILABLE]

23. 5,830,721, Nov. 3, 1998, DNA mutagenesis by random fragmentation andreassembly; Willem P. C. Stemmer, et al., 435/489, 6, 91.2 [IMAGE AVAILABLE]

24. 5,821,352, Oct. 13, 1998, CDNA collections encoding proteins regulated during programmed cell death, methods of preparation thereof and methods of use thereof; Nathaniel Heintz, et al., 536/23.5, 24.31 [IMAGE AVAILABLE]

L13 1. 5,874,424, Feb. 23, 1999, Inhibitors of interleukin-1.beta. converting enzyme; Mark James Batchelor, et al., 514/221, 211, 220; 540/491, 495, 500, 501 [IMAGE AVAILABLE]

2. 5,861,498, Jan. 19, 1999, Nucleotides encoding immunophilin FKBP46 and fragments thereof; Emad S. Alnemri, et al., 536/23.5; 435/320.1 [IMAGE AVAILABLE]

3. 5,858,778, Jan. 12, 1999, SF caspase-1 and compositions for making and methods of using the same; Emad S. Alnemri, et al., 435/325, 219, 252.3, 252.33, 254.11, 320.1, 348; 536/23.2, 24.3, 24.31 [IMAGE AVAILABLE]

4. 5,851,815, Dec. 22, 1998, MCH4 and MCH5, apoptotic proteases; Emad S. Alnemri, et al., 435/219, 183, 212; 530/324 [IMAGE AVAILABLE]

5. 5,843,904, Dec. 1, 1998, Inhibitors of interleukin-1.beta.converting enzyme; Guy W. Bemis, et al., 514/18, 19; 530/330, 331 [IMAGE AVAILABLE]

6. 5,834,228, Nov. 10, 1998, Method for identifying inhibitors for apopain based upon the crystal structure of the apopain: Ac-DEVD-CHO complex; Joseph W. Becker, et al., 435/23, 212 [IMAGE AVAILABLE]

7. 5,786,173, Jul. 28, 1998, MCH4 and MCH5, apoptotic protease, nucleic acids encoding and methods of use; Emad S. Alnemri, et al., 435/69.1, 70.1, 183, 219, 252.3, 320.1; 530/324, 350; 536/23.1, 23.5, 24.31 [IMAGE AVAILABLE]

514/221, 211, 220; 540/491, 495, 500, 501 [IMAGE AVAILABLE]

5. 5,869,519, Feb. 9, 1999, C-terminal modified (n-substituted)-2- indolyl dipeptides as inhibitors of the **ICE**/ced-3 **family** of cysteine proteases; Donald S. Karanewsky, et al., 514/415, 385, 419; 548/312.1, 492, 493 [IMAGE AVAILABLE]

6. 5,869,315, Feb. 9, 1999, Modified **interleukin**-1.beta. **converting** enzyme with increased stability; Robert V. Talanian, et al., 435/226, 23, 41, 184, 219; 530/351; 536/23.5 [IMAGE AVAILABLE]

7. 5,861,268, Jan. 19, 1999, Method for induction of tumor cell **apoptosis** with chemical inhibitors targeted to 12-lipoxygenase; Dean G. Tang, et al., 435/25, 4, 183, 975 [IMAGE AVAILABLE]

8. 5,858,778, Jan. 12, 1999, SF caspase-1 and compositions for making and methods of using the same; Emad S. Alnemri, et al., 435/325, 219, 252.3, 252.33, 254.11, 320.1, 348; 536/23.2, 24.3, 24.31 [IMAGE AVAILABLE]

9. 5,858,678, Jan. 12, 1999, **Apoptosis**-regulating proteins; Govindaswamy Chinnadurai, 435/7.1, 365; 530/328, 329, 350; 536/23.5 [IMAGE AVAILABLE]

10. 5,858,669, Jan. 12, 1999, Beclin, a novel bcl-2 interacting gene near BRCA1 on chromosome 17q21 and methods of use; Beth Cindy Levine, 435/6 [IMAGE AVAILABLE]

11. 5,856,169, Jan. 5, 1999, Isoforms of human **interleukin**-1.beta. **converting** enzyme and methods of using the same; Gerald Litwack, et al., 435/252.3, 320.1, 325; 536/23.2, 24.31, 24.33 [IMAGE AVAILABLE]

12. 5,856,116, Jan. 5, 1999, Crystal structure and mutants of **interleukin**-1 beta **converting** enzyme; Keith P. Wilson, et al., 435/23, 24, 212, 219, 226 [IMAGE AVAILABLE]

13. 5,851,815, Dec. 22, 1998, MCH4 and MCH5, **apoptotic** proteases; Emad S. Alnemri, et al., 435/219, 183, 212; 530/324 [IMAGE AVAILABLE]

14. 5,847,135, Dec. 8, 1998, Inhibitors of **interleukin**-1.beta. **converting** enzyme; Guy W. Bemis, et al., 544/264; 540/1, 450, 470, 476, 486, 500, 519, 553; 544/1, 179, 180, 224, 355, 383, 386; 546/112, 146, 162, 192, 245, 272.4, 272.7, 273.7, 275.4, 279.1; 548/183, 200, 304.4, 333.5, 482, 494, 504, 571 [IMAGE AVAILABLE]

15. 5,846,768, Dec. 8, 1998, Invertebrate **apoptosis** gene `GRIM` and methods of producing the protein encoded thereby; John M. Abrams, et al., 435/69.1, 252.3, 320.1, 325, 348; 536/23.1, 23.5, 24.1, 24.3, 24.33 [IMAGE AVAILABLE]

16. 5,843,904, Dec. 1, 1998, Inhibitors of **interleukin**-1.beta.**converting** enzyme; Guy W. Bemis, et al., 514/18, 19; 530/330, 331 [IMAGE AVAILABLE]

17. 5,843,659, Dec. 1, 1998, **Apoptosis** gene EI24, compositions, and methods of use; Sophie M. Lehar, et al., 435/6, 69.1, 91.4, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]

18. 5,840,509, Nov. 24, 1998, Protease and related nucleic acid compounds; Binhui Ni, et al., 435/7.35; 424/139.1, 150.1, 164.1; 530/327, 387.9, 388.2, 388.4, 389.5, 391.3, 391.7 [IMAGE AVAILABLE]

19. 5,837,838, Nov. 17, 1998, Bax inhibitor proteins; John C. Reed, et al., 536/23.1; 530/350 [IMAGE AVAILABLE]

20. 5,837,837, Nov. 17, 1998, Nucleic acids molecules encoding Caspase-8h and Caspase-8i; John J. Hunter, et al., 536/23.1; 530/300, 350 [IMAGE AVAILABLE]